Connecting via Winsock to STN

Welcome to STN International! Enter x:x

LOGINID:SSSPTA1644PNH

PASSWORD:
TERMINAL (ENTER 1, 2, 3, OR ?):2

* * * * * * * * * * Welcome to STN International * * * * * * * * * *

NEWS 1		Web Page URLs for STN Seminar Schedule - N. America
NEWS 2	Jan 25	BLAST(R) searching in REGISTRY available in STN on the Web
NEWS 3	Jan 29	FSTA has been reloaded and moves to weekly updates
NEWS 4	Feb 01	DKILIT now produced by FIZ Karlsruhe and has a new update frequency
NEWS 5	Feb 19	Access via Tymnet and SprintNet Eliminated Effective 3/31/02
NEWS 6	Mar 08	Gene Names now available in BIOSIS
NEWS 7	Mar 22	TOXLIT no longer available
NEWS 8	Mar 22	TRCTHERMO no longer available
NEWS 9	Mar 28	US Provisional Priorities searched with P in CA/CAplus and USPATFULL
NEWS 10	Mar 28	LIPINSKI/CALC added for property searching in REGISTRY
NEWS 11	Apr 02	PAPERCHEM no longer available on STN. Use PAPERCHEM2 instead.
NEWS 12	Apr 08	"Ask CAS" for self-help around the clock
NEWS 13	Apr 09	BEILSTEIN: Reload and Implementation of a New Subject Area
NEWS 14	Apr 09	ZDB will be removed from STN
NEWS 15	Apr 19	US Patent Applications available in IFICDB, IFIPAT, and IFIUDB
NEWS 16	Apr 22	Records from IP.com available in CAPLUS, HCAPLUS, and ZCAPLUS
NEWS 17	Apr 22	BIOSIS Gene Names now available in TOXCENTER
NEWS 18	Apr 22	Federal Research in Progress (FEDRIP) now available
NEWS 19	Jun 03	New e-mail delivery for search results now available
NEWS 20	Jun 10	MEDLINE Reload
NEWS 21	Jun 10	PCTFULL has been reloaded

NEWS EXPRESS	February 1 CURRENT WINDOWS VERSION IS V6.0d, CURRENT MACINTOSH VERSION IS V6.0a(ENG) AND V6.0Ja(JP), AND CURRENT DISCOVER FILE IS DATED 05 FEBRUARY 2002
NEWS HOURS	STN Operating Hours Plus Help Desk Availability
NEWS INTER	General Internet Information
NEWS LOGIN	Welcome Banner and News Items
NEWS PHONE	Direct Dial and Telecommunication Network Access to STN
NEWS WWW	CAS World Wide Web Site (general information)

Enter NEWS followed by the item number or name to see news on that specific topic.

All use of STN is subject to the provisions of the STN Customer agreement. Please note that this agreement limits use to scientific research. Use for software development or design or implementation of commercial gateways or other similar uses is prohibited and may result in loss of user privileges and other penalties.

* * * * * * * * * * * * * * * STN Columbus * * * * * * * * * * * * * * *

FILE 'HOME' ENTERED AT 09:31:40 ON 01 JUL 2002

```
=> file medline embase biosis scisearch caplus
COST IN U.S. DOLLARS
                                                SINCE FILE      TOTAL
                                                   ENTRY    SESSION
FULL ESTIMATED COST                                 0.21       0.21

FILE 'MEDLINE' ENTERED AT 09:32:05 ON 01 JUL 2002

FILE 'EMBASE' ENTERED AT 09:32:05 ON 01 JUL 2002
COPYRIGHT (C) 2002 Elsevier Science B.V.  All rights reserved.

FILE 'BIOSIS' ENTERED AT 09:32:05 ON 01 JUL 2002
COPYRIGHT (C) 2002 BIOLOGICAL ABSTRACTS INC.(R)

FILE 'SCISEARCH' ENTERED AT 09:32:05 ON 01 JUL 2002
COPYRIGHT (C) 2002 Institute for Scientific Information (ISI) (R)

FILE 'CAPLUS' ENTERED AT 09:32:05 ON 01 JUL 2002
USE IS SUBJECT TO THE TERMS OF YOUR STN CUSTOMER AGREEMENT.
PLEASE SEE "HELP USAGETERMS" FOR DETAILS.
COPYRIGHT (C) 2002 AMERICAN CHEMICAL SOCIETY (ACS)

=> s ganglioside
L1        51040 GANGLIOSIDE

=> s l1 and modulat?
L2         2708 L1 AND MODULAT?

=> s l2 and agent
L3          219 L2 AND AGENT

=> s l3 and treatment
L4          105 L3 AND TREATMENT

=> s l4 and allergy
L5            1 L4 AND ALLERGY

=> d l5 cbib abs

L5   ANSWER 1 OF 1  CAPLUS  COPYRIGHT 2002 ACS
2001:798033   Document No. 135:356779  Artificial antigen-presenting cells for
      manipulation of antigen-specific T-cells.  Albani, Salvatore (USA).  PCT
      Int. Appl. WO 2001080833 A1 20011101, 195 pp.  DESIGNATED STATES: W:  AE,
      AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, DM,
      DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG,
      KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO,
      NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG,
      US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE,
      BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT,
      LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG.  (English).  CODEN: PIXXD2.
      APPLICATION: WO 2000-IT161 20000420.
AB    The authors discloses the prepn. and characterization of artificial
      antigen-presenting cells.  Thesee artificial antigen-presenting cells may
      be used in isolating and expanding T-cell populations as well as
      modulating T-cell responses.  In several examples, the author
      discloses methods for the construction of liposomes contg. MHC-peptide
      complexes, accessory mols., co-stimulatory mols., and adhesion mols.  In
      addn., the liposome can contain other mols. irrelevant to T-cell binding
      or modulation that are used in binding of these artificial
      antigen-presenting cells to solid support systems that may be used in the
      retrieval and identification of antigen-specific T-cells.  Addnl., the
      present invention is directed to devices and methods for treating
      conditions which would benefit from modulation of T-cell
```

response, for example, autoimmune disorders, **allergies**, cancers, viral infections, and graft rejection.

=> d his

(FILE 'HOME' ENTERED AT 09:31:40 ON 01 JUL 2002)

FILE 'MEDLINE, EMBASE, BIOSIS, SCISEARCH, CAPLUS' ENTERED AT 09:32:05 ON 01 JUL 2002

L1 51040 S GANGLIOSIDE
L2 2708 S L1 AND MODULAT?
L3 219 S L2 AND AGENT
L4 105 S L3 AND TREATMENT
L5 1 S L4 AND ALLERGY

=> s l4 adn hypersensitivity
MISSING OPERATOR L4 ADN
The search profile that was entered contains terms or nested terms that are not separated by a logical operator.

=> s l4 and hypersensitivity
L6 6 L4 AND HYPERSENSITIVITY

=> dup remove l6
PROCESSING COMPLETED FOR L6
L7 2 DUP REMOVE L6 (4 DUPLICATES REMOVED)

=> d l7 1-2 cbib abs

L7 ANSWER 1 OF 2 MEDLINE DUPLICATE 1
2001384810 Document Number: 21331901. PubMed ID: 11438174. Brain-derived **gangliosides** suppress the chronic relapsing-remitting experimental autoimmune encephalomyelitis in NOD mice induced with myelin oligodendrocyte glycoprotein peptide. Sekiguchi Y; Ichikawa M; Inoue A; Itoh M; Koh C S. (Department of Pediatrics, Shinshu University School of Medicine, 3-1-1 Asahi, 390-8621, Matsumoto, Japan.) JOURNAL OF NEUROIMMUNOLOGY, (2001 Jun 1) 116 (2) 196-205. Journal code: 8109498. ISSN: 0165-5728. Pub. country: Netherlands. Language: English.

AB Chronic relapsing-remitting experimental autoimmune encephalomyelitis (CREAE) induced with myelin oligodendrocyte glycoprotein peptides 35-55 (MOG(35-55)) in NOD mice was successfully treated with brain-derived **gangliosides** (GA). The GA **treatment** suppressed the development and severity of CREAE, both clinically and histologically. Spleen cells from the GA-treated mice displayed markedly inhibited levels of MOG(35-55) specific proliferation and interferon-gamma production. Delayed-type **hypersensitivity** reactions to MOG(35-55) were suppressed by the GA **treatment**. GA **modulate** various T cell effector functions in CREAE and may be an effective therapeutic **agent** for autoimmune demyelinating diseases such as multiple sclerosis.

L7 ANSWER 2 OF 2 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
1992:304297 Document No.: BA94:17447. **GANGLIOSIDE MODULATION** OF CD4 DOES NOT BLOCK T-HELPER CELL FUNCTION AS COMPARED TO ANTAGONISM BY ANTI-CD4 ANTIBODY. MORRISON W J; OFFNER H; VANDENBARK A A. VETERANS ADM. MED. CENT. 151-DD, 3710 S.W. U.S. VETERANS HOSPITAL ROAD, PORTLAND, OREGON 97207.. DRUG DEV RES, (1992) 25 (4), 315-323. CODEN: DDREDK. ISSN: 0272-4391. Language: English.

AB **Gangliosides** (GM1) **modulate** CD4 from the surface of T-helper lymphocytes and have been shown to block CD4-mediated HIV-1 infection in vitro. However, the effects of GM1 on CD4 are neutralized in the presence of serum. We sought to make a GM1 derivative which would

resist serum neutralization and to determine whether **modulation** of CD4 by GM1 would affect T-helper lymphocyte function. Study of GM1 and GM1-derivatives in the presence of serum indicated that the active conformation involves intact sialic acid hydroxyl groups and intermolecular **ganglioside** interactions. All chemical modifications directed toward the sialic acid moiety reduced the ability of GM1 to **modulate** CD4. Elution profiles of GM1 in the presence and absence of albumin indicate that the active form of GM1 is represented by micelles that are disrupted by albumin. Function studies show that CD4 T-helper cells made CD- by GM1 **treatment** continued to proliferate in culture and recognize antigen (Ag) through the T cell receptor (TcR). Moreover, T-helper cell mediated inflammation assessed by Ag-specific, delayed type **hypersensitivity** (DTH) was enhanced after passive transfer of GM1-treated T-helper cells to naive animals. In comparison, CD4 blockade by anti-CD4 antibody decreased Ag-stimulated T-helper cell proliferation and DTH reactions. These results demonstrate that 1) CD4 **modulation** involves an active conformation that arises from sialic acid effector molecules organized by micellar GM1 conformations; and 2) the mechanism by which GM1 **modulates** CD4 is different from CD4 antagonism seen with anti-CD4 antibody **treatment**.

```
=> s "Etx"
L8          766 "ETX"

=> s l8 and ganglioside
L9            8 L8 AND GANGLIOSIDE

=> dup remove l9
PROCESSING COMPLETED FOR L9
L10           4 DUP REMOVE L9 (4 DUPLICATES REMOVED)

=> d l10 1-4 cbib abs

L10  ANSWER 1 OF 4  CAPLUS  COPYRIGHT 2002 ACS
2001:798749   Document No. 135:339267  Therapeutic agents.  Williams, Neil
     Andrew; Hirst, Timothy Raymond; Nashar, Toufic Osman (UK).  U.S. Pat.
     Appl. Publ. US 20010036917 A1 20011101, 53 pp., Cont.-in-part of U.S.
     6,287,563.  (English).  CODEN: USXXCO.  APPLICATION: US 2001-867914
     20010530.  PRIORITY: GB 1995-13733 19950705; US 1997-999458 19971229.
AB   A method of treating diabetes in a mammalian subject by administering an
     agent capable of modulating a ganglioside GM-1 (GM-1) assocd.
     activity in an amt. effect to treat the disease; wherein agent is selected
     from the group consisting of cholera toxin (Ctx), enterotoxins (
     Etx), the B subunit of Ctx and the B subunit of Etx,
     mutants and derivs. thereof. along with co-administration of antigens
     which are not so linked to form a single active agent.

L10  ANSWER 2 OF 4  CAPLUS  COPYRIGHT 2002 ACS
1999:36021   Document No. 130:164241  Receptor mediated apoptosis of CD8+T
     cells by the B subunits of cholera-like enterotoxins.  Pitman, Richard S.;
     Hirst, Timothy R.; Nashar, Toufic O.; Williams, Neil A. (Department of
     Pathology and Microbiology, School of Medical Sciences, University of
     Bristol, Bristol, BS8 1TD, UK).  Biochemical Society Transactions, 26(4),
     S338 (English) 1998.  CODEN: BCSTB5.  ISSN: 0300-5127.  Publisher:
     Portland Press Ltd..
AB   Heat-labile enterotoxin (Etx) B subunit (EtxB) and cholera toxin
     (Ctx) B subunit directly mediate apoptosis of CD8+T cells through an
     interaction with GM1, present on lymphocyte cell surfaces.  Although the
     precise signaling pathways which mediate EtxB induced cellular activation
     and apoptosis remain unknown, it has been demonstrated that resp. levels
     of ceramide and MAPK (mitogen-activated protein kinase) activity remain
```

unaltered in both T and B lymphocytes upon addn. of EtxB, thereby excluding a role for these signaling mechanisms.

L10 ANSWER 3 OF 4 MEDLINE DUPLICATE 1
96324796 Document Number: 96324796. PubMed ID: 8671661. Cross-linking of cell surface **ganglioside** GM1 induces the selective apoptosis of mature CD8+ T lymphocytes. Nahar T O; Williams N A; Hirst T R. (Research School of Biosciences, University of Kent, Canterbury, UK.) INTERNATIONAL IMMUNOLOGY, (1996 May) 8 (5) 731-6. Ref: 24. Journal code: 8916182. ISSN: 0953-8178. Pub. country: ENGLAND: United Kingdom. Language: English.

AB **Gangliosides** are glycosphingolipids found ubiquitously on the surface of mammalian cells. They contain a ceramide tail that is inserted into the membrane and exposed carbohydrate and sialic acid moieties. The non-toxic B subunit oligomer (EtxB) of Escherichia coli heat-labile enterotoxin (**Etx**) is a potent immunogen in vivo and has profound modulatory effects on EtxB-primed lymphocytes in vitro, properties which are dependent on its ability to bind to GM1 **ganglioside** receptors. Here, it is shown that cross-linking GM1 by EtxB causes a differential effect on mature CD4(+) and CD8(+) T cells from lymph node cultures proliferating in response to an unrelated antigen, ovalbumin. Addition of EtxB to such cultures led to the complete depletion of CD8(+) T cells compared with enhanced activation of CD4(+) cells [as measured by expression of CD25 (IL-2Ralpha)]. By contrast, addition of a mutant EtxB, EtxB(G33D), which does not bind to GM1, failed to trigger CD8(+) T cell depletion. When EtxB was added to isolated non-immune CD8(+) lymphocytes rapid (12-18 h) alterations in nuclear morphology and the appearance of sub-G0/G1 levels of DNA were induced; properties which are characteristic of cells undergoing apoptosis. EtxB(G33D) failed to trigger apoptosis, indicating that the induction of the apoptotic signal was dependent on the binding of GM1. These findings provide an insight into the potent immunogenicity and immunomodulatory properties of E. coli enterotoxins as well as heralding a novel method for the selective induction of apoptosis in mature CD8(+) T lymphocytes.

L10 ANSWER 4 OF 4 SCISEARCH COPYRIGHT 2002 ISI (R)
94:247507 The Genuine Article (R) Number: NG675. PURIFICATION OF THE B-SUBUNIT OLIGOMER OF ESCHERICHIA-COLI HEAT-LABILE ENTEROTOXIN BY HETEROLOGOUS EXPRESSION AND SECRETION IN A MARINE VIBRIO. AMIN T; HIRST T R (Reprint). UNIV KENT, BIOL LAB, CANTERBURY CT2 7NJ, KENT, ENGLAND (Reprint); UNIV KENT, BIOL LAB, CANTERBURY CT2 7NJ, KENT, ENGLAND. PROTEIN EXPRESSION AND PURIFICATION (APR 1994) Vol. 5, No. 2, pp. 198-204. ISSN: 1046-5928. Pub. country: ENGLAND. Language: ENGLISH.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Heat-labile enterotoxins (**Etx**) are plasmid-encoded, multimeric proteins produced by certain diarrheagenic strains of Eschenichia coli. The nontoxic, receptor-binding B subunit (EtxB) of such toxins may be useful as a component of vaccines against enterotoxigenic E. coli, or as a carrier for the delivery of heterologous epitopes to the mucosal immune system. Here we describe a simple method for the purification of EtxB from a marine vibrio harboring a broad-host range controlled expression vector containing the etxB gene. Induction of EtxB resulted in its specific secretion to the medium, to a concentration of greater than 25 mg/liter of culture. The techniques of ultrafiltration and hydrophobic interaction chromatography were used to purify EtxB to homogeneity from the medium of this organism (with a yield of 60.7%). EtxB-epitope fusion proteins were also successfully expressed and secreted in this marine vibrio, suggesting that this system may be of general use in the preparation of EtxB-based vaccines. (C) 1991 Academic Press,Inc.

=> s "Ctx"
L11 9952 "CTX"

```
=> s l11 adn ganglioside
MISSING OPERATOR L11 ADN
The search profile that was entered contains terms or
nested terms that are not separated by a logical operator.

=> s l11 and ganglioside
L12          64 L11 AND GANGLIOSIDE

=> s l12 unconjugate
MISSING OPERATOR L12 UNCONJUGATE
The search profile that was entered contains terms or
nested terms that are not separated by a logical operator.

=> s l12 and hypersensitivity
L13           0 L12 AND HYPERSENSITIVITY

=> s l12 and IgE
L14           0 L12 AND IGE

=> s l12 and allergy
L15           0 L12 AND ALLERGY

=> s l12 and mutant
L16           1 L12 AND MUTANT

=> d l16 cbib abs

L16  ANSWER 1 OF 1  CAPLUS  COPYRIGHT 2002 ACS
2001:798749   Document No. 135:339267  Therapeutic agents.  Williams, Neil
     Andrew; Hirst, Timothy Raymond; Nashar, Toufic Osman (UK).  U.S. Pat.
     Appl. Publ. US 20010036917 A1 20011101, 53 pp., Cont.-in-part of U.S.
     6,287,563.  (English).  CODEN: USXXCO.  APPLICATION: US 2001-867914
     20010530.  PRIORITY: GB 1995-13733 19950705; US 1997-999458 19971229.
AB   A method of treating diabetes in a mammalian subject by administering an
     agent capable of modulating a ganglioside GM-1 (GM-1) assocd.
     activity in an amt. effect to treat the disease; wherein agent is selected
     from the group consisting of cholera toxin (Ctx), enterotoxins
     (Etx), the B subunit of Ctx and the B subunit of Etx,
     mutants and derivs. thereof. along with co-administration of
     antigens which are not so linked to form a single active agent.


=> d his

     (FILE 'HOME' ENTERED AT 09:31:40 ON 01 JUL 2002)

     FILE 'MEDLINE, EMBASE, BIOSIS, SCISEARCH, CAPLUS' ENTERED AT 09:32:05 ON
     01 JUL 2002
L1         51040 S GANGLIOSIDE
L2          2708 S L1 AND MODULAT?
L3           219 S L2 AND AGENT
L4           105 S L3 AND TREATMENT
L5             1 S L4 AND ALLERGY
L6             6 S L4 AND HYPERSENSITIVITY
L7             2 DUP REMOVE L6 (4 DUPLICATES REMOVED)
L8           766 S "ETX"
L9             8 S L8 AND GANGLIOSIDE
L10            4 DUP REMOVE L9 (4 DUPLICATES REMOVED)
L11         9952 S "CTX"
L12           64 S L11 AND GANGLIOSIDE
L13            0 S L12 AND HYPERSENSITIVITY
L14            0 S L12 AND IGE
L15            0 S L12 AND ALLERGY
```

```
L16             1 S L12 AND MUTANT

=> dup remove l64
L64 IS NOT VALID HERE
The L-number entered has not been defined in this session, or it
has been deleted.  To see the L-numbers currently defined in this
session, enter DISPLAY HISTORY at an arrow prompt (=>).

=> d his

     (FILE 'HOME' ENTERED AT 09:31:40 ON 01 JUL 2002)

     FILE 'MEDLINE, EMBASE, BIOSIS, SCISEARCH, CAPLUS' ENTERED AT 09:32:05 ON
     01 JUL 2002
L1          51040 S GANGLIOSIDE
L2           2708 S L1 AND MODULAT?
L3            219 S L2 AND AGENT
L4            105 S L3 AND TREATMENT
L5              1 S L4 AND ALLERGY
L6              6 S L4 AND HYPERSENSITIVITY
L7              2 DUP REMOVE L6 (4 DUPLICATES REMOVED)
L8            766 S "ETX"
L9              8 S L8 AND GANGLIOSIDE
L10             4 DUP REMOVE L9 (4 DUPLICATES REMOVED)
L11          9952 S "CTX"
L12            64 S L11 AND GANGLIOSIDE
L13             0 S L12 AND HYPERSENSITIVITY
L14             0 S L12 AND IGE
L15             0 S L12 AND ALLERGY
L16             1 S L12 AND MUTANT

=> dup remove l12
PROCESSING COMPLETED FOR L12
L17            20 DUP REMOVE L12 (44 DUPLICATES REMOVED)

=> d l17 1-20 cbib abs

L17  ANSWER 1 OF 20     MEDLINE                                  DUPLICATE 1
2002187774  Document Number: 21918066.    PubMed ID: 11918702.
     Cholesterol-rich plasma membrane domains (lipid rafts) in keratinocytes:
     importance in the baseline and UVA-induced generation of reactive oxygen
     species. Gniadecki Robert; Christoffersen Nanna; Wulf Hans Christian.
     (Department of Dermatology, University of Copenhagen, Bispebjerg Hospital,
     Copenhagen, Denmark.. rgniadecki@hotmail.com) . JOURNAL OF INVESTIGATIVE
     DERMATOLOGY, (2002 Apr) 118 (4) 582-8.  Journal code: 0426720. ISSN:
     0022-202X. Pub. country: United States. Language: English.
AB   The biologic effects of ultraviolet radiation such as DNA damage,
     mutagenesis, cellular aging, and carcinogenesis are in part mediated by
     reactive oxygen species. In unirradiated cells the major known sources of
     reactive oxygen species are the mitochondrial respiratory chain and the
     membrane oxidases functionally coupled to several membrane growth factor
     receptors. There is evidence that mitochondria also play a role in
     oxidative stress after ultraviolet irradiation; however, it is unknown
     whether the biochemical processes at the level of the plasma membrane
     contribute to the regulation of reactive oxygen species synthesis. In
     order to elucidate this issue we examined here the importance of the
     microdomain plasma membrane organization in the regulation of oxidative
     stress in unirradiated and ultraviolet A (340-400 nm) irradiated HaCaT
     keratinocytes. Labeling of confluent HaCaT cultures with fluorescently
     tagged cholera toxin B subunit (FITC-CTx) revealed the presence
     of GM1 ganglioside and cholesterol-rich microdomains (lipid
     rafts) that formed junction-like structures in the membranes of adjacent
     cells and patchy microdomains elsewhere. There was a marked heterogeneity
```

in the level of FITC-**CTx** labeling: there were groups of cells demonstrating prominent labeling (FITC-**CTx**(high)) whereas other cells were only weakly labeled (FITC-**CTx**(low)). When reactive oxygen species synthesis was measured with the fluorescent probe carboxy-2',7'-dichlorodihydrofluorescein diacetate, we found that (i) the baseline and ultraviolet-A-induced reactive oxygen species synthesis correlated with the magnitude of FITC-**CTx** labeling and was highest in the FITC-**CTx**(high) cells; (ii) reactive oxygen species synthesis was diminished in cells in which the integrity of membrane domains was disrupted by cholesterol sequestration with methyl-beta-cyclodextrin and filipin, or after treatment with GM1 **ganglioside**; (iii) reactive oxygen species synthesis in cholesterol-depleted cells was fully restored after cholesterol repletion. We conclude that the plasma membrane takes part in the regulation of oxidative stress in keratinocytes and disruption of its microdomain structure reduces reactive oxygen species synthesis both at the baseline and after ultraviolet A irradiation. We hypothesize that lipid-raft-associated protein(s) may be involved in the generation of reactive oxygen species and that pharmacologic modulation of membrane structure may provide a novel therapeutic approach relevant for photoprotection and cutaneous carcinogenesis.

L17 ANSWER 2 OF 20 CAPLUS COPYRIGHT 2002 ACS

2001:798749 Document No. 135:339267 Therapeutic agents. Williams, Neil Andrew; Hirst, Timothy Raymond; Nashar, Toufic Osman (UK). U.S. Pat. Appl. Publ. US 20010036917 A1 20011101, 53 pp., Cont.-in-part of U.S. 6,287,563. (English). CODEN: USXXCO. APPLICATION: US 2001-867914 20010530. PRIORITY: GB 1995-13733 19950705; US 1997-999458 19971229.

AB A method of treating diabetes in a mammalian subject by administering an agent capable of modulating a **ganglioside** GM-1 (GM-1) assocd. activity in an amt. effect to treat the disease; wherein agent is selected from the group consisting of cholera toxin (**Ctx**), enterotoxins (Etx), the B subunit of **Ctx** and the B subunit of Etx, mutants and derivs. thereof. along with co-administration of antigens which are not so linked to form a single active agent.

L17 ANSWER 3 OF 20 MEDLINE DUPLICATE 2

2001195617 Document Number: 21104214. PubMed ID: 11160430. Membrane lipid rafts are necessary for the maintenance of the (alpha)7 nicotinic acetylcholine receptor in somatic spines of ciliary neurons. Bruses J L; Chauvet N; Rutishauser U. (Cellular Biochemistry and Biophysics Program, Memorial Sloan-Kettering Cancer Center, New York, New York 10021, USA.) JOURNAL OF NEUROSCIENCE, (2001 Jan 15) 21 (2) 504-12. Journal code: 8102140. ISSN: 1529-2401. Pub. country: United States. Language: English.

AB Calcium-permeable neurotransmitter receptors are concentrated into structurally and biochemically isolated cellular compartments to localize calcium-mediated events during neurotransmission. The cytoplasmic membrane contains lipid microdomains called lipid rafts, which can gather into microscopically visible clusters, and thus the association of a particular protein with lipid rafts can result in its redistribution on the cell surface. The present study asks whether lipid rafts participate in the formation and maintenance of the calcium-permeable alpha7-subunit nicotinic acetylcholine receptor (alpha7nAChR) clusters found in somatic spines of ciliary neurons. Lipid rafts and alpha7nAChR become progressively colocalized within somatic spines during synaptogenesis. To determine whether these rafts are required for the maintenance of alpha7nAChR aggregates, cholesterol was extracted from dissociated ciliary neurons by treatment with methyl-beta-cyclodextrin. This treatment caused the dispersion of lipid rafts and the redistribution of alpha7nAChR into small clusters over the cell surface, suggesting that the integrity of lipid rafts is required to maintain the receptor clustering. However, lipid raft dispersion also caused the depolymerization of the F-actin cytoskeleton, which can also tether the receptor at specific sites. To

assess whether interaction between rafts and alpha7nAChR is independent of F-actin filaments, the lipid raft patches were stabilized with a combination of the cholera toxin B subunit (**CTX**), which specifically binds to the raft component **ganglioside** GM1, and an antibody against **CTX**. The stabilized rafts were then treated with latrunculin-A to depolymerize F-actin. Under these conditions, large patches of **CTX** persisted and were colocalized with alpha7nAChR, indicating that the aggregates of receptors can be maintained independently of the underlying F-actin cytoskeleton. Moreover, it was found that the alpha7nAChR is resistant to detergent extraction at 4 degrees C and floats with the caveolin-containing lipid-rich fraction during density gradient centrifugation, properties that are consistent with a direct association between the receptor and the membrane microdomains.

L17 ANSWER 4 OF 20 MEDLINE DUPLICATE 3

2001206192 Document Number: 21124873. PubMed ID: 11222115. Peroral immunization with Helicobacter pylori adhesin protein genetically linked to cholera toxin A2B subunits. Kim B O; Shin S S; Yoo Y H; Pyo S. (School of Pharmacy, Sung Kyun Kwan University, Suwon, 440-746, Kyunggi-Do, South Korea.) CLINICAL SCIENCE, (2001 Mar) 100 (3) 291-8. Journal code: 7905731. ISSN: 0143-5221. Pub. country: England: United Kingdom. Language: English.

AB Helicobacter pylori is a major cause of gastric-associated diseases. To evaluate the efficacy of a possible vaccine antigen against H. pylori infection, the chimaeric construct adhesin--CTXA2B, derived from H. pylori adhesin genetically coupled to cholera toxin (**CTX**) subunits A2 and B (CTXA2B), was expressed in Escherichia coli as an insoluble recombinant chimaeric protein. The protein was then purified by denaturation, renaturation and size-exclusion chromatography. The composition of purified adhesin--CTXA2B was verified by SDS/PAGE and Western blotting with antibodies to antigenic components of adhesin and CTXB, and confirmed as a chimaeric protein with G(M1)-**ganglioside** binding activity and adhesin epitopes by a G(M1)-ELISA developed using antibodies to adhesin. Oral immunization of mice with adhesin--CTXA2B induced higher levels of mucosal IgA and serum IgG antibodies to H. pylori adhesin and to CTXB than in mice immunized with adhesin or CTXA2B alone. Adhesin--CTXA2B was also demonstrated to be a potential protective antigen in a mouse model of H. pylori infection. The immunization of mice with adhesin--CTXA2B protected 62.5% of mice infected with H. pylori SS1 strain, whereas adhesin immunization was not able to confer protection to mice. This protection may be correlated with high levels of mucosal IgA and serum IgG antibodies against H. pylori adhesin. Taken together, the results indicate that the genetically linked CTXA2B acts as a useful mucosal adjuvant, and that the adhesin-CTXA2B chimaeric protein could be a potential component in future H. pylori vaccine development.

L17 ANSWER 5 OF 20 MEDLINE DUPLICATE 4

2001639659 Document Number: 21547852. PubMed ID: 11689159. Cholera toxin-B subunit blocks excitatory opioid receptor-mediated hyperalgesic effects in mice, thereby unmasking potent opioid analgesia and attenuating opioid tolerance/dependence. Shen K F; Crain S M. (Department of Neuroscience, Albert Einstein College of Medicine, Yeshiva University, 1300 Morris Park Ave. Bronx, NY 10461, USA.) BRAIN RESEARCH, (2001 Nov 16) 919 (1) 20-30. Journal code: 0045503. ISSN: 0006-8993. Pub. country: Netherlands. Language: English.

AB In a previous study we demonstrated that injection (i.p.) of low doses of GM1 **ganglioside** in mice rapidly attenuates morphine's analgesic effects. This result is consonant with our electrophysiologic studies in nociceptive types of dorsal root ganglion (DRG) neurons in culture, which showed that exogenous GM1 rapidly increased the efficacy of excitatory (Gs-coupled) opioid receptor functions. By contrast, treatment of DRG neurons with the non-toxic B-subunit of cholera toxin (**CTX**-B)

which binds selectively to GM1, blocked the excitatory, but not inhibitory, effects of morphine and other bimodally-acting opioid agonists, thereby resulting in a net increase in inhibitory opioid potency. The present study provides more direct evidence that endogenous GM1 plays a physiologic role in regulating excitatory opioid receptor functions in vivo by demonstrating that cotreatment with remarkably low doses of **CTX**-B (10 ng/kg, s.c.) selectively blocks hyperalgesic effects elicited by morphine or by a kappa opioid agonist, thereby unmasking potent opioid analgesia. These results are comparable to the effects of cotreatment of mice with morphine plus an ultra-low dose of the opioid antagonist, naltrexone (NTX) which blocks opioid-induced hyperalgesic effects, unmasking potent opioid analgesia. Low-dose NTX selectively blocks excitatory opioid receptors at their recognition site, whereas **CTX**-B binds to, and interferes with, a putative allosteric GM1 regulatory site on excitatory opioid receptors. Furthermore, chronic cotreatment of mice with morphine plus **CTX** -B attenuates development of opioid tolerance and physical dependence, as previously shown to occur during cotreatment with low-dose NTX.

L17 ANSWER 6 OF 20 MEDLINE DUPLICATE 5
2001062223 Document Number: 20414047. PubMed ID: 10959489. Endogenous GM1 **ganglioside** of the plasma membrane promotes neuritogenesis by two mechanisms. Fang Y; Wu G; Xie X; Lu Z H; Ledeen R W. (New Jersey Medical School, UMDNJ, Department of Neurosciences, Newark, 07103, USA.) NEUROCHEMICAL RESEARCH, (2000 Jul) 25 (7) 931-40. Journal code: 7613461. ISSN: 0364-3190. Pub. country: United States. Language: English.

AB The influence of GM1 on the neuritogenic phase of neuronal differentiation has been highlighted in recent reports showing upregulation of this **ganglioside** in the plasma and nuclear membranes concomitant with axonogenesis. These changes are accompanied by alterations in Ca2+ flux which constitute an essential component of the signaling mechanism for axon outgrowth. This study examines 2 distinct mechanisms of induced neurite outgrowth involving plasma membrane GM1, as expressed in 3 neuroblastoma cell lines. Growth of Neuro-2a and NG108-15 cells in the presence of neuraminidase (N'ase), an enzyme that increases the cell surface content of GM1, caused prolific outgrowth of neurites which, in the case of Neuro-2a, could be blocked by the B subunit of cholera toxin (**Ctx** B) which binds specifically to GM1; however, the latter agent applied to NG108-15 cells proved neuritogenic and potentiated the effect of N'ase. With N18 cells, the combination was also neuritogenic as was **Ctx** B alone, whereas N'ase by itself had no effect. Neurite outgrowth correlated with influx of extracellular Ca2+, determined with fura-2. Treatment of NG108-15 and N18 cells with **Ctx** B alone caused modest but persistent elevation of intracellular Ca2+ while a more pronounced increase occurred with the combination **Ctx** B + N'ase. Treatment with N'ase alone also caused modest but prolonged elevation of intracellular Ca2+ in NG108-15 and Neuro-2a but not N18; in the case of Neuro-2a this effect was blocked by **Ctx** B. Neuro-2a and N18 thus possess 2 distinctly different mechanisms for neuritogenesis based on Ca2+ modulation by plasma membrane GM1, while NG108-15 cells show both capabilities. The neurites stimulated by N'ase + **Ctx** B treatment of N18 cells were shown to have axonal character, as previously demonstrated for NG108-15 cells stimulated in this manner and for Neuro-2a cells stimulated by N'ase alone.

L17 ANSWER 7 OF 20 SCISEARCH COPYRIGHT 2002 ISI (R)
2000:847874 The Genuine Article (R) Number: 371DC. Different stimulatory opioid effects on intracellular Ca2+ in SH-SY5Y cells. Chen L Y (Reprint); Zou S B; Lou X L; Kang H G. HUAZHONG UNIV SCI & TECHNOL, INST BIOCHEM & BIOPHYS, WUHAN 430074, PEOPLES R CHINA (Reprint). BRAIN RESEARCH (3 NOV 2000) Vol. 882, No. 1-2, pp. 256-265. Publisher: ELSEVIER SCIENCE BV. PO BOX 211, 1000 AE AMSTERDAM, NETHERLANDS. ISSN: 0006-8993. Pub. country: PEOPLES R CHINA. Language: English.

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Present study revealed the stimulatory effects of delta opioid receptor on intracellular Ca2+ concentration ([Ca2+](i)) in SH-SY5Y cells. Fura-2 based single cell fluorescence ratio (F345/F380) was used to monitor the fluctuation of [Ca2+](i). Application of the selective delta-opioid receptor agonist alone, [D-Pen(2.5)]-enkephalin (DPDPE), hardly had any effects on cells cultivated for 3-10 days. However, after the cells had been pre-stimulated with cholinoceptor agonist, carbachol, variable calcium elevation was found in 59% of the cultures. The response was naltridole-reversible and dose-dependent, and was abolished completely by thapsigargin (TG) treatment but not by administration of CdCl2 or 0-Ca2+ bath solutions. DPDPE-mediated [Ca2+](i) elevation was abolished by pertussis toxin (PTX) pretreatment but not cholera toxin (**CTX**), indicating coupling via G proteins of G(i)/G(o) subfamily. In 17.5% of the responding cells, biphase response was found which may be due to both the stimulatory and the inhibitory effects of opioid. On the other hand, in acutely dissociated cells, DPPDE alone induced [Ca2+](i) increase in 50% of the cultures. The probability and the amplitude of the elevation were decreased considerably by application of nifedipine or 0-Ca2+ bath solution and was little affected by application of TG. DPDPE activated [Ca2+](i) increase via a PTX insensitive and **CTX**-sensitive pathway suggesting coupling through G(s) subunit. All these indicated the opioid modulated the intracellular Ca2+ regulation system through different pathways. SH-SY5Y cell line might be a suitable model for the investigation of the complex mechanism which underlies opioid function. (C) 2000 Elsevier Science B.V. All rights reserved.

L17 ANSWER 8 OF 20 SCISEARCH COPYRIGHT 2002 ISI (R)

1999:811804 The Genuine Article (R) Number: 247HG. Heterogeneous expression of voltage-gated potassium channels of the shaker family (Kv1) in oligodendrocyte progenitors. Schmidt K; Eulitz D; Veh R W; Kettenmann H; Kirchhoff F (Reprint). MAX DELBRUCK CTR MOL MED, ROBERT ROSSLE STR 10, D-13092 BERLIN, GERMANY (Reprint); MAX DELBRUCK CTR MOL MED, D-13092 BERLIN, GERMANY; HUMBOLDT UNIV, MED SCH CHARITE, DEPT ANAT, D-10098 BERLIN, GERMANY. BRAIN RESEARCH (2 OCT 1999) Vol. 843, No. 1-2, pp. 145-160. Publisher: ELSEVIER SCIENCE BV. PO BOX 211, 1000 AE AMSTERDAM, NETHERLANDS. ISSN: 0006-8993. Pub. country: GERMANY. Language: English.

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Outwardly rectifying K+ channels determine the membrane conductance and influence the proliferation rate of glial progenitor cells. To analyte the molecular identity and the functional role of K+ channels in glial progenitors of mouse brain, expression of shaker-type Kv1 genes was studied at three levels: (1) presence of Kv1 mRNAs, (2) biosynthesis of channel proteins and (3) electrophysiological and pharmacological properties of K+ currents. mRNA expression of Kv1.1 to Kv1.6 genes was studied by single-cell reverse transcription-mediated polymerase chain reaction (RT-PCR) using degenerate primers to amplify the six Kv1 transcripts. Most cells expressed several mRNA combinations simultaneously. In more than half of the cells, messages for Kv1.2, Kv1.5 and Kv1.6 were found, while Kv1.1, Kv1.3 and Kv1.4 were detected in only a minority of cells. In contrast, at the level of protein expression - employing immunocytochemistry with subtype-specific antibodies - Kv1.2 and Kv1.3 were undetectable (< 2%), while almost all cells expressed Kv1.4 (85%), Kv1.5 (99%) and Kv1.6 (99%). Kv1.1 was present in a minor cell population (10%). Functional contribution of Kv1 proteins to progenitor membrane conductance was determined by analyzing the voltage-dependence of K+ current activation and inactivation as well as their current sensitivities to the subtype-preferring blockers and toxins tetraethylammonium (TEA), 4-aminopyridine (4-AP), charybdoroxin (**CTX**), alpha-dendrotoxin (DTX) and mast-cell degranulating peptide (MCDP). From these results, it is concluded: first, glial progenitor cells can express all transcripts of the six Kv1 genes, but do not express all proteins; second, Kv1.4, Kv1.5 and Kv1.6 proteins are most abundant and

were found in the majority of cells; and third, K+ currents flow predominantly either through heteromeric channel complexes or through homomeric Kv1.5 ion pores, but not through homomeric Kv1.4 or Kv1.6 channels. (C) 1999 Elsevier Science B.V. All rights reserved.

L17 ANSWER 9 OF 20 CAPLUS COPYRIGHT 2002 ACS

1999:36021 Document No. 130:164241 Receptor mediated apoptosis of CD8+T cells by the B subunits of cholera-like enterotoxins. Pitman, Richard S.; Hirst, Timothy R.; Nashar, Toufic O.; Williams, Neil A. (Department of Pathology and Microbilogy, School of Medical Sciences, University of Bristol, Bristol, BS8 1TD, UK). Biochemical Society Transactions, 26(4), S338 (English) 1998. CODEN: BCSTB5. ISSN: 0300-5127. Publisher: Portland Press Ltd..

AB Heat-labile enterotoxin (Etx) B subunit (EtxB) and cholera toxin (**Ctx**) B subunit directly mediate apoptosis of CD8+T cells through an interaction with GM1, present on lymphocyte cell surfaces. Although the precise signaling pathways which mediate EtxB induced cellular activation and apoptosis remain unknown, it has been demonstrated that resp. levels of ceramide and MAPK (mitogen-activated protein kinase) activity remain unaltered in both T and B lymphocytes upon addn. of EtxB, thereby excluding a role for these signaling mechanisms.

L17 ANSWER 10 OF 20 SCISEARCH COPYRIGHT 2002 ISI (R)

97:266242 The Genuine Article (R) Number: WQ103. Cholera toxin effects on body temperature changes induced by morphine. Basilico L; Parenti M; Fumagalli A; Parolaro D; Giagnoni G (Reprint). UNIV MILAN, FAC SCI, INST PHARMACOL, VIA VANVITELLI 32-A, I-20129 MILAN, ITALY (Reprint); UNIV MILAN, FAC SCI, INST PHARMACOL, I-20129 MILAN, ITALY; UNIV MILAN, SCH MED, DEPT PHARMACOL, I-20129 MILAN, ITALY. PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR (MAR 1997) Vol. 56, No. 3, pp. 499-505. Publisher: PERGAMON-ELSEVIER SCIENCE LTD. THE BOULEVARD, LANGFORD LANE, KIDLINGTON, OXFORD, ENGLAND OX5 1GB. ISSN: 0091-3057. Pub. country: ITALY. Language: English.

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB The present study evaluates the influence of cholera toxin and its B-subunit on thermic responses to morphine in the rats. The holotoxin (1 mu g/rat) and the B-subunit (5 mu g) were administered ICV and three days later rats were challenged ICV with morphine and tested for changes of body temperature. Cholera toxin, but not its B-subunit, modified the time course of the hyperthermic response induced by a low dose of morphine (2.5 mu g), converted the hypothermia due to a higher dose of morphine (18 mu g) to a consistent hyperthermia and only partially I:educed the greater hypothermia induced by 36 mu g of morphine. Cholera toxin-induced modifications of thermic responses to morphine were paralleled with a decreased G(s alpha) immunoreactivity and a reduced ability for the toxin to catalyse the ''in vitro'' ADP-ribosylation of G(s alpha) in hypothalamic membranes. In contrast, at the same time when morphine-induced effects on body temperature were assessed, no changes in pertussis toxin-mediated ADP-ribosylation of G(i alpha)/G(o alpha), or basal adenylate cyclase activity, or binding of mu-opioid receptor selective ligand [H-3]-DAMGO were observed in hypothalamic areas from rats treated with cholera toxin. These findings suggest that adaptative events secondary to prolonged activation of G(s alpha) play a role in the modifications of thermic responses to morphine induced by **CTX**. Copyright (C) 1997 Elsevier Science Inc.

L17 ANSWER 11 OF 20 MEDLINE DUPLICATE 6

96302630 Document Number: 96302630. PubMed ID: 8723763. Trophic effect of cholera toxin B subunit in cultured cerebellar granule neurons: modulation of intracellular calcium by GM1 **ganglioside**. Wu G; Lu Z H; Nakamura K; Spray D C; Ledeen R W. (Department of Neurosciences, New Jersey Medical School of UMDNJ, Newark 07103, USA.) JOURNAL OF NEUROSCIENCE RESEARCH, (1996 May 1) 44 (3) 243-54. Journal code: 7600111.

ISSN: 0360-4012. Pub. country: United States. Language: English.

AB Survival of cerebellar granule cells (CGC) in culture was significantly improved in the presence of cholera toxin B subunit (**Ctx** B), a ligand which binds to GM1 with specificity and high affinity. This trophic effect was linked to elevation of intracellular calcium ([Ca2+]i), and was additive to that of high K+. Survival was optimized when **Ctx** B was present for several days during the early culture period. 45Ca2+ and cell survival studies indicated the mechanism to involve enhanced influx of Ca2+ through L-type voltage-sensitive channels, since the trophic effect was blocked by antagonists specific for that channel type. Inhibitors of N-methyl-D-aspartate receptor/channels were without effect. During the early stage of culture **Ctx** B, together with 25 mM K+, caused [Ca2+]i to rise to 0.2-0.7 microM in a higher proportion of cells than 25 mM K+ alone. A significant change in the nature of GM1 modulation of Ca2+ flux occurred after 7 days in culture, at which time **Ctx** B ceased to elevate and instead reduced [Ca2+]i below the level attained with 25 mM K+. GM1 thus appears to serve as intrinsic inhibitor of one or more L-type Ca2+ channels during the first 7 days in vitro, and then as intrinsic activator of (possibly other) L-type channels after that period. This is the first demonstration of a modulatory role for GM1 **ganglioside** affecting Ca2+ homeostasis in cultured neurons of the CNS.

L17 ANSWER 12 OF 20 SCISEARCH COPYRIGHT 2002 ISI (R)

95:753960 The Genuine Article (R) Number: TB490. DUAL REGULATION BY MU-OPIOID, DELTA-OPIOID AND KAPPA-OPIOID RECEPTOR AGONISTS OF K+ CONDUCTANCE OF DRG NEURONS AND NEUROBLASTOMA X DRG NEURON HYBRID F11 CELLS . FAN S F; CRAIN S M (Reprint). YESHIVA UNIV ALBERT EINSTEIN COLL MED, DEPT NEUROSCI, 1300 MORRIS PK AVE, BRONX, NY, 10461 (Reprint); YESHIVA UNIV ALBERT EINSTEIN COLL MED, DEPT NEUROSCI, BRONX, NY, 10461; YESHIVA UNIV ALBERT EINSTEIN COLL MED, DEPT PHYSIOL BIOPHYS, BRONX, NY, 10461; SUNY STONY BROOK, HLTH SCI CTR, DEPT PHYSIOL & BIOPHYS, STONY BROOK, NY, 11794. BRAIN RESEARCH (23 OCT 1995) Vol. 696, No. 1-2, pp. 97-105. ISSN: 0006-8993. Pub. country: USA. Language: ENGLISH.

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB The effects of the mu opioid receptor agonists, morphine and Tyr-D-Ala-Gl-N-methyl-Phe-Gl-ol (DAGO), the delta opioid receptor agonist, Tyr-D-Pen-Gly-Phe-D-penicillamine (DPDPE) and the kappa-opioid receptor agonist, dynorphin A-(1-13) on the whole-cell K+ currents (I-K) of cultured mouse DRG neurons and neuroblastoma X DRG neuron hybrid F11 cells were studied. These opioid ligands all elicited dual effects. Low concentrations (< nM) usually elicited a transient increase in I-K (within 1 min), followed by a sustained decrease in I-K. In contrast, mu M concentrations rapidly elicited a sustained increase in I-K. After brief treatment with cholera toxin subunit B (**CTX**-B), the usual sustained decrease in I-K evoked by < nM opioid agonists no longer occurred. Low concentrations then elicited only a sustained increase in I-K. On the other hand, after chronic treatment with pertussis toxin (PTX), the usual mu M opioid-induced increases in I-K no longer occurred and more than half of the cells responded with a sustained decrease of I-K to mu M as well as nM opioids. The results suggest that mu, delta and kappa opioid receptors are each coupled to K+ channels through **CTX** -B- and PTX-sensitive transduction systems. Both systems have similar threshold concentrations to opioids. Activation of the **CTX** -B-sensitive opioid receptor/transduction system resulted in a decrease in K+ conductance of the cell which is generally associated with an increase in neuronal excitability. Activation of the other system resulted in an increase in K+ conductance which will, in general, decrease neuronal excitability. The net change in the I-K depends upon which effect predominates. The dominance at different opioid concentrations may depend on the relative efficacies of the coupling of these two systems to K+ channels.

L17 ANSWER 13 OF 20 MEDLINE

94335720 Document Number: 94335720. PubMed ID: 8057923. Regulation of cholera toxin by temperature, pH, and osmolarity. Gardel C L; Mekalanos J J. (Department of Microbiology and Molecular Genetics, Harvard Medical School, Boston, Massachusetts 02115.) METHODS IN ENZYMOLOGY, (1994) 235 517-26. Ref: 40. Journal code: 0212271. ISSN: 0076-6879. Pub. country: United States. Language: English.

L17 ANSWER 14 OF 20 MEDLINE DUPLICATE 7

93019059 Document Number: 93019059. PubMed ID: 1383408. Vaccination by cholera toxin conjugated to a herpes simplex virus type 2 glycoprotein D peptide. Drew M D; Estrada-Correa A; Underdown B J; McDermott M R. (Department of Pathology, McMaster University, Hamilton, Ontario, Canada.) JOURNAL OF GENERAL VIROLOGY, (1992 Sep) 73 (Pt 9) 2357-66. Journal code: 0077340. ISSN: 0022-1317. Pub. country: ENGLAND: United Kingdom. Language: English.

AB Immunization of BALB/cJ mice with a peptide corresponding to residues 1 to 23 of glycoprotein D [gD(1-23)] from herpes simplex virus type 2 (HSV-2) elicits antibody responses which correlate with protection against lethal HSV-2 infection. In the present study, we examined the ability of cholera toxin (**CTX**) to act as an immunogenic carrier for gD(1-23). The number of gD(1-23) residues conjugated to **CTX** affected its binding to GM1 **ganglioside** and physiological toxicity, both of which are factors affecting oral immunogenicity. The antibody response elicited after intraperitoneal (i.p.) immunization with the **CTX** -gD(1-23) conjugate was protective against a lethal i.p. challenge with HSV-2. In other experiments, mice were immunized i.p. on day 0 and subsequent immunizations conducted on days 14 and 28 were administered either intragastrically or intravaginally (i.vag.). Intraperitoneal priming followed by either i.p or intragastric boosting resulted in anti-HSV-2 antibodies in vaginal washings and in protection against a lethal i.vag. challenge with HSV-2. Intraperitoneal priming followed by i.vag. boosting did not elicit anti-HSV-2 antibodies in vaginal washings and did not protect mice against a lethal i.vag. challenge with HSV-2. These results suggest that **CTX** can act as a systemic and an oral delivery molecule for the covalently linked gD(1-23) peptide and that such conjugates can elicit protective immune responses against systemic and genital HSV-2 infection.

L17 ANSWER 15 OF 20 MEDLINE DUPLICATE 8

92370332 Document Number: 92370332. PubMed ID: 1324084. After chronic opioid exposure sensory neurons become supersensitive to the excitatory effects of opioid agonists and antagonists as occurs after acute elevation of GM1 **ganglioside**. Crain S M; Shen K F. (Department of Neuroscience, Albert Einstein College of Medicine, Yeshiva University, Bronx, NY 10461.) BRAIN RESEARCH, (1992 Mar 13) 575 (1) 13-24. Journal code: 0045503. ISSN: 0006-8993. Pub. country: Netherlands. Language: English.

AB Mouse sensory dorsal-root ganglion (DRG) neurons chronically exposed to 1 microM D-Ala2-D-Leu5-enkephalin (DADLE) for greater than 1 week in culture become tolerant to opioid inhibitory effects, i.e. shortening of the duration of the calcium-dependent component of the action potential (APD). Acute application of higher concentrations of DADLE (ca. 10 microM) to these treated neurons not only fails to shorten the APD but, instead, generally elicits excitatory effects, i.e. prolongation of the APD. The present study shows that chronic DADLE- or morphine-treated DRG neurons also become supersensitive to the excitatory effects of opioids. Whereas nM concentrations of dynorphin(1-13) are generally required to prolong the APD of naive DRG neurons, fM levels become effective after chronic opioid treatment. Whereas 1-30 nM naloxone or diprenorphine do not alter the APD of naive DRG neurons, both opioid antagonists unexpectedly prolong the APD of most of the treated cells. Similar supersensitivity to the excitatory effects of opioid agonists and antagonists was previously observed after

acute treatment of naive DRG neurons with GM1 **ganglioside**. Our results suggest that both chronic opioid and acute GM1 treatments of DRG neurons greatly enhance the efficacy of opioid excitatory receptor functions so that even the extremely weak agonist properties of naloxone and diprenorphine become effective in prolonging the APD of these treated cells when tested at low concentrations, whereas their antagonist properties at inhibitory opioid receptors do not appear to be altered. Furthermore, whereas cholera toxin-B subunit (**CTX**-B; 1-10 nM) blocks opioid-induced APD prolongation in naive DRG neurons (presumably by interfering with endogenous GM1 modulation of excitatory opioid receptors functions), even much higher concentrations of **CTX**-B were ineffective in chronic opioid-treated as well as acute GM1-elevated neurons. These and related data suggest that opioid excitatory supersensitivity in chronic opioid-treated DRG neurons may be due to a cyclic AMP-dependent increase in GM1 **ganglioside** levels. Our results may clarify mechanisms of opioid dependence and the paradoxical supersensitivity to naloxone which triggers withdrawal symptoms after opiate addiction.

L17 ANSWER 16 OF 20 MEDLINE DUPLICATE 9

92145433 Document Number: 92145433. PubMed ID: 1685937. Brief treatment of sensory ganglion neurons with GM1 **ganglioside** enhances the efficacy of opioid excitatory effects on the action potential. Shen K F; Crain S M; Ledeen R W. (Department of Neuroscience, Albert Einstein College of Medicine, Yeshiva University, Bronx, NY 10461.) BRAIN RESEARCH, (1991 Sep 13) 559 (1) 130-8. Journal code: 0045503. ISSN: 0006-8993. Pub. country: Netherlands. Language: English.

AB In previous studies, we showed that low (nM) concentrations of opioid prolong the action potential duration (APD) of many mouse dorsal root ganglion (DRG) neurons via Gs-linked excitatory opioid receptors, whereas micromolar opioid levels shorten the APD via Gi/Go-linked inhibitory receptors. In addition, cholera toxin-B subunit (**CTX**-B) selectively blocks opioid- but not forskolin-induced prolongation of the APD in DRG neurons. Since **CTX**-B binds with selective high affinity to GM1 **ganglioside** located on the cell surface, the results suggest that GM1 plays an essential role in regulating excitatory opioid receptor functions. This hypothesis was tested by treating DRG neurons in mouse DRG-cord explants with exogenous **gangliosides** and determining whether the efficacy of opioid agonists in prolonging the APD is enhanced. The threshold concentration of the opioids, dynorphin(1-13) and morphine required to prolong the APD in many DRG neurons was markedly decreased from nM to fM levels after bath exposure to 10 nM to 1 microM GM1 **ganglioside** for less than 5 min. In contrast, GM2 and GM3 **gangliosides** and asialo-GM1 **ganglioside** were ineffective, even when DRG neurons were exposed to high concentrations (1-10 microM) for periods greater than 1 h. Although GD1a, GD1b and GQ1b **gangliosides** appeared to be as effective as GM1 when tested at microM concentrations for 15 min, tests at lower concentrations, shorter periods, and/or at lower temperature (24 degrees vs 34 degrees C), showed that they were significantly less effective than GM1.(ABSTRACT TRUNCATED AT 250 WORDS)

L17 ANSWER 17 OF 20 MEDLINE DUPLICATE 10

91372242 Document Number: 91372242. PubMed ID: 1654261. Cholera toxin and Gs protein modulation of synaptic transmission in guinea pig mesenteric artery. Nozaki M; Sperelakis N. (Department of Physiology and Biophysics, University of Cincinnati, College of Medicine, OH 45267-0576.) EUROPEAN JOURNAL OF PHARMACOLOGY, (1991 May 2) 197 (1) 57-62. Journal code: 1254354. ISSN: 0014-2999. Pub. country: Netherlands. Language: English.

AB Cholera toxin (**CTX**) was used to test whether the presynaptic beta-adrenoceptors of guinea-pig mesenteric artery are coupled via stimulatory GTP-binding proteins. The vascular smooth muscle cells were electrically quiescent unless stimulated and had a mean resting potential

of -68.7 +/- 2.8 mV (n = 16) and input resistance of 12.1 +/- 0.5 M omega (n = 4). Perivascular nerve stimulation with brief square pulses evoked excitatory junction potentials (EJPs) in the muscle cells. Isoproterenol (0.1 microM) enhanced the EJP amplitude without modifying the passive membrane properties of the muscle cells. The beta-blocker, propranolol (0.5 microM), prevented the effects of isoproterenol on EJP amplitude. The permeant analogue of cyclic AMP, 8-bromocAMP, also potentiated EJP amplitude. EJP amplitude was markedly enhanced by treatment of the isolated blood vessels with **CTX** (10 micrograms/ml for 1 h). The muscle cells became hyperpolarized (-74.6 +/- 2.1 mV, n = 5), and their input resistances were significantly reduced (8.2 +/- 0.5 M omega, n = 4). These effects of **CTX** persisted after washout. Addition of GM1 **ganglioside** (5 micrograms/ml) prevented the **CTX** effects. The **CTX** enhancement of EJP amplitude was not prevented by application of depolarizing current (ca. 0.5 nA) the muscle cells (to counter the hyperpolarization). These results suggest that **CTX** increases the neurotransmitter release from the nerve terminals; the hyperpolarization may be due to an increase in K+ conductance. These effects of **CTX** may be mainly due to elevation of cAMP in the nerve terminal and in the muscle cell.

L17 ANSWER 18 OF 20 MEDLINE DUPLICATE 11

91287564 Document Number: 91287564. PubMed ID: 2099384. Biological and immunological characterization of a cloned cholera toxin-like enterotoxin from Salmonella typhimurium. Prasad R; Chopra A K; Peterson J W; Pericas R; Houston C W. (Department of Microbiology, University of Texas Medical Branch, Galveston 77550-2782.) MICROBIAL PATHOGENESIS, (1990 Nov) 9 (5) 315-29. Journal code: 8606191. ISSN: 0882-4010. Pub. country: ENGLAND: United Kingdom. Language: English.

AB A chromosomal DNA fragment, encoding an enterotoxin gene of Salmonella typhimurium Q1, was cloned into bacteriophage EMBL3 and plasmid vector pBR322. The recombinant clones lambda B8 and pC1 were identified using a synthetic oligonucleotide probe made to the B subunit region of the cholera toxin gene (**ctx**). Cell lysates of Escherichia coli VCS257 [lambda B8] induced fluid secretion in rabbit intestinal loops, while lysates of E. coli DH5 alpha [pC1] failed to elicit an enterotoxic response in this model. Both lysates and partially purified preparations elongated Chinese hamster ovary (CHO) cells, elevated cellular cAMP and PGE2, and bound to **ganglioside** GM1. The biological activity associated with the cloned enterotoxin was neutralized by monospecific antiserum to cholera toxin (CT). Immunoblots of pC1 and lambda B8 lysates probed with anti-CT, exhibited a 30 kDa protein similar to that of pJM17, which carried the **ctx** gene. Under non-dissociating conditions, anti-CT immunoblots of the same lysates revealed two proteins, one corresponding in size to the holotoxin and the other to CT-A. When analysed by DNA-directed protein synthesis in vitro, both pC1 and lambda B8 DNA expressed two unique proteins (30 and 11 kDa) similar to that of pJM17.

L17 ANSWER 19 OF 20 MEDLINE DUPLICATE 12

91145662 Document Number: 91145662. PubMed ID: 1981160. Cholera toxin-B subunit blocks excitatory effects of opioids on sensory neuron action potentials indicating that GM1 **ganglioside** may regulate Gs-linked opioid receptor functions. Shen K F; Crain S M. (Department of Neuroscience, Albert Einstein College of Medicine, Yeshiva University, Bronx, NY 10461.) BRAIN RESEARCH, (1990 Oct 29) 531 (1-2) 1-7. Journal code: 0045503. ISSN: 0006-8993. Pub. country: Netherlands. Language: English.

AB In a previous study, we demonstrated that cholera toxin-A subunit, as well as the whole toxin, selectively blocks opioid-induced prolongation of the Ca2+ component of the action potential duration (APD) in dorsal root ganglion (DRG) neurons, indicating mediation of this excitatory effect by Gs-linked opioid receptors. The present study shows that pretreatment of

DRG neurons with the B subunit of cholera toxin (1-10 ng/ml; greater than 15 min) can also block mu/delta and kappa opioid-induced APD prolongation, but not shortening. Since the B subunit binds selectively to GM1 **ganglioside** located on the cell surface, these results suggest that this **ganglioside** may regulate Gs-linked excitatory opioid receptor functions in DRG neurons. Possible contamination of purified B subunit preparations of cholera toxin with traces of the more potent A subunit was eliminated by heating the stock solution to 56 degrees C for 20 min. Exposure of DRG neurons to an affinity-purified anti-GM1 antiserum also blocked opioid-induced APD prolongation, providing further evidence that GM1 **ganglioside** may play an essential role in excitatory opioid modulation of the action potential of these cells. The blockade by cholera toxin-B subunit and anti-GM1 antibodies of opioid-induced APD prolongation is best accounted for by the following hypothesis: **CTX**-B interferes with an endogenous GM1 **ganglioside** component of the excitatory, but not inhibitory, opioid receptor complex on DRG neurons that may allosterically regulate coupling of the receptors via Gs to adenylate cyclase/cyclic adenosine monophosphate-dependent ionic conductances.

L17 ANSWER 20 OF 20 CAPLUS COPYRIGHT 2002 ACS
1977:51310 Document No. 86:51310 Studies on anti-cholinesterase activity of cobra cardiotoxin. Shiau Lin, Shoei-Yn; Liao, Chin; Lee, C. Y. (Coll. Med., Natl. Taiwan Univ., Taipei, Taiwan). T'ai-wan I Hsueh Hui Tsa Chih, 75(8), 440-8 (English) 1976. CODEN: TIHHAH.
AB Cobra (Naja naja atra) venom was fractionated on a column of CM-Sephadex C 50 into 12 fractions by gradient elution with ammonium acetate buffer. Only the 3 cardiotoxic fractions (fractions X, XI and XII) exhibited anti-cholinesterase (ChE) [9001-08-5] activity. This anti-ChE activity of cardiotoxin (**CTX**) was inhibited not only by MgCl2, CaCl2 or univalent cations, but also by polyaninons (heparin [9005-49-6], **ganglioside** and RNA). The anti-ChE activity was destroyed completely by heating at 100.degree. for 10 min at pH 8.0 but not at pH 5.0 even with prolonged heating for 60 min. Mg2+ reversed the anti-ChE activity of **CTX** but not that of cobra venom. The ash of cobra venom as well as ZnCl2 possessed not only irreversible anti-ChE activity but also converted the reversible **CTX** anti-ChE activity into an irreversible one. Polylysine [25104-18-1] as well as protamine mimicked anti-ChE activity of **CTX**, but neither lysine, arginine nor spermidine, spermine possessed this activity. Apparently, **CTX** inactivated ChE by ionic binding of its basic groups with anionic sites of ChE mols. and inorg. cations interfere with their interaction. Because of such an interference, no anti-ChE effects were obsd. with **CTX** in any biol. system.

=> s "CtxB"
L18 338 "CTXB"

=> s l18 and allergy
L19 1 L18 AND ALLERGY

=> d l19 cbib abs

L19 ANSWER 1 OF 1 CAPLUS COPYRIGHT 2002 ACS
2000:814337 Document No. 133:361908 Bacteriophage isolated from bacterial genomes and extrachromosomal elements and methods of use thereof. Karaolis, David K. R. (University of Maryland, Baltimore, USA). PCT Int. Appl. WO 2000067784 A1 20001116, 59 pp. DESIGNATED STATES: W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US,

UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 2000-US12580 20000510. PRIORITY: US 1999-PV133373 19990510.

AB The present invention relates to compns., methods, processes, etc., relating to bacteriophage which are encoded by chromosome, plasmids, or an extrachromosomal element of bacteria. The bacteriophage of the present invention are preferably encoded by pathogenicity islands in chromosomes or plasmids of pathogenic bacteria. The bacteriophage can be utilized as a pharmaceutical compn., e.g., to elicit an immune response, e.g., for the purpose of producing antibodies, as vaccines and vaccine vectors to regulate the immune system, e.g., for the prevention and treatment of **allergy**, disease, and other pathol. conditions. The invention finds addnl. utility in systems and methods for the detection of pathogens comprising bacteriophage and a system and method for the environmental eradication of pathogenic microorganisms.

=> d his

(FILE 'HOME' ENTERED AT 09:31:40 ON 01 JUL 2002)

FILE 'MEDLINE, EMBASE, BIOSIS, SCISEARCH, CAPLUS' ENTERED AT 09:32:05 ON 01 JUL 2002

```
L1         51040 S GANGLIOSIDE
L2          2708 S L1 AND MODULAT?
L3           219 S L2 AND AGENT
L4           105 S L3 AND TREATMENT
L5             1 S L4 AND ALLERGY
L6             6 S L4 AND HYPERSENSITIVITY
L7             2 DUP REMOVE L6 (4 DUPLICATES REMOVED)
L8           766 S "ETX"
L9             8 S L8 AND GANGLIOSIDE
L10            4 DUP REMOVE L9 (4 DUPLICATES REMOVED)
L11         9952 S "CTX"
L12           64 S L11 AND GANGLIOSIDE
L13            0 S L12 AND HYPERSENSITIVITY
L14            0 S L12 AND IGE
L15            0 S L12 AND ALLERGY
L16            1 S L12 AND MUTANT
L17           20 DUP REMOVE L12 (44 DUPLICATES REMOVED)
L18          338 S "CTXB"
L19            1 S L18 AND ALLERGY
```

```
=> dup remove l18
PROCESSING COMPLETED FOR L18
L20          128 DUP REMOVE L18 (210 DUPLICATES REMOVED)

=> s l20 and asthma
L21            0 L20 AND ASTHMA

=> d l20 1-128 cbib abs
```

L20 ANSWER 1 OF 128 CAPLUS COPYRIGHT 2002 ACS

2002:465849 Therapeutic agent comprising a b-subunit of a protein toxin. Morgan, Andrew John; Wilson, Andrew Douglas; Ong, Kong Wee (University of Bristol, UK). PCT Int. Appl. WO 2002047727 A1 20020620, 49 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN,

YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2001-GB5452 20011211. PRIORITY: GB 2000-30067 20001211.

AB A B-subunit of a protein toxin selected from the B-subunit ofE. coli heat-labile enterotoxin (EtxB) and the B-subunit of Vibrio cholerae toxin (**CtxB**) has a therapeutic effect against cell surface-expressed viral antigens and tumor antigens. In particular, the protein toxin may be used to treat an animal body, including human, suffering from a disease or condition assocd. with Epstein Barr Virus or suffering from neoplasia. The therapeutic agent may, addnl., comprise a cell surface-expressed antigen, for instance an Epstein Barr Virus latent membrane protein.

L20 ANSWER 2 OF 128 MEDLINE DUPLICATE 1

2002253086 Document Number: 21988181. PubMed ID: 11877421. A kinetic model of intermediate formation during assembly of cholera toxin B-subunit pentamers. Lesieur Claire; Cliff Matthew J; Carter Rachel; James Roger F L; Clarke Anthony R; Hirst Timothy R. (Department of Pathology, School of Medical Sciences University of Bristol, Bristol, United Kingdom.) JOURNAL OF BIOLOGICAL CHEMISTRY, (2002 May 10) 277 (19) 16697-704. Journal code: 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.

AB Cholera toxin is the most important virulence factor produced by Vibrio cholerae. The pentameric B-subunit of the toxin can bind to GM1-ganglioside receptors, leading to toxin entry into mammalian cells. Here, the in vitro disassembly and reassembly of **CtxB**(5) (the B subunit pentamer of cholera toxin) is investigated. When **CtxB**(5) was acidified at pH 1.0 and then neutralized, the B-subunits disassembled and could no longer migrate as SDS-stable pentamers on polyacrylamide gels or be captured by GM1. However, continued incubation at neutral pH resulted in the B-subunits regaining the capacity to be detected by GM1 enzyme-linked immunosorbent assay (t(12) approximately 8 min) and to migrate as SDS-stable pentamers (t(12) approximately 15 min). Time-dependent changes in Trp fluorescence intensity during B-subunit reassembly occurred with a half-time of approximately 8 min, similar to that detected by GM1 enzyme-linked immunosorbent assay, suggesting that both methods monitor earlier events than B-pentamer formation alone. Based on the Trp fluorescence intensity measurements, a kinetic model of the pathway of **CtxB**(5) reassembly was generated that depended on trans to cis isomerization of Pro-93 to give an interface capable of subunit-subunit interaction. The model suggests formation of intermediates in the reaction, and these were successfully detected by glutaraldehyde cross-linking.

L20 ANSWER 3 OF 128 MEDLINE DUPLICATE 2

2002294632 Document Number: 22031180. PubMed ID: 12034098. Comparison of mucosal and systemic humoral immune responses after transcutaneous and oral immunization strategies. John Manohar; Bridges Emily A; Miller Andy O; Calderwood Stephen B; Ryan Edward T. (Tropical & Geographic Medicine Center, Division of Infectious Diseases, Jackson 504, Massachusetts General Hospital, 55 Fruit Street, 02114, Boston, MA, USA.) VACCINE, (2002 Jun 21) 20 (21-22) 2720-6. Journal code: 8406899. ISSN: 0264-410X. Pub. country: England: United Kingdom. Language: English.

AB In order to compare the ability of transcutaneous and oral immunization strategies to induce mucosal and systemic immune responses, we inoculated mice transcutaneously with cholera toxin (CT) or the non-toxic B subunit of cholera toxin (**CtxB**), or orally with Peru2(pETR1), an attenuated vaccine strain of Vibrio cholerae expressing **CtxB**. In addition, we also evaluated dual immunization regimens (oral inoculation with transcutaneous boosting, and transcutaneous immunization with oral boosting) in an attempt to optimize induction of both mucosal and systemic immune responses. We found that transcutaneous immunization with purified **CtxB** or CT induces much more prominent systemic IgG anti-**CtxB** responses than does oral inoculation with a vaccine vector

strain of V. cholerae expressing **CtxB**. In comparison, anti-**CtxB** IgA in serum, stool and bile were comparable in mice either transcutaneously or orally immunized. Overall, the most prominent systemic and mucosal anti-**CtxB** responses occurred in mice that were orally primed with Peru2(pETR1) and transcutaneously boosted with CT. Our results suggest that combination oral and transcutaneous immunization strategies may most prominently induce both mucosal and systemic humoral responses.

L20 ANSWER 4 OF 128 MEDLINE DUPLICATE 3

2002239677 Document Number: 21950561. PubMed ID: 11953381. Evidence for the emergence of non-O1 and non-O139 Vibrio cholerae strains with pathogenic potential by exchange of O-antigen biosynthesis regions. Li Manrong; Shimada Toshio; Morris J Glenn Jr; Sulakvelidze Alexander; Sozhamannan Shanmuga. (Department of Epidemiology and Preventive Medicine, University of Maryland School of Medicine, Baltimore, Maryland 21201, USA.) INFECTION AND IMMUNITY, (2002 May) 70 (5) 2441-53. Journal code: 0246127. ISSN: 0019-9567. Pub. country: United States. Language: English.

AB The novel epidemic strain Vibrio cholerae O139 Bengal originated from a seventh-pandemic O1 El Tor strain by antigenic shift resulting from homologous recombination-mediated exchange of O-antigen biosynthesis (wb*) clusters. Conservation of the genetic organization of wb* regions seen in other serogroups raised the possibility of the existence of pathogenic non-O1 and non-O139 V. cholerae strains that emerged by similar events. To test this hypothesis, 300 V. cholerae isolates of non-O1 and non-O139 serogroups were screened for the presence of virulence genes and an epidemic genetic background by DNA dot blotting, IS1004 fingerprinting, and restriction fragment length polymorphism (RFLP) analysis. We found four non-O1 strains (serogroups O27, O37, O53, and O65) with an O1 genetic backbone suggesting exchange of wb* clusters. DNA sequence analysis of the O37 wb* region revealed that a novel approximately 23.4-kb gene cluster had replaced all but the approximately 4.2-kb right junction of the 22-kb O1 wbe region. In sharp contrast to the backbones, the virulence regions of the four strains were quite heterogeneous; the O53 and O65 strains had the El Tor vibrio pathogenicity island (VPI) cluster, the O37 strain had the classical VPI cluster, and the O27 strain had a novel VPI cluster. Two of the four strains carried CTXphi; the O27 strain possessed a CTXphi with a recently reported immune specificity (rstR-4** allele) and a novel **ctxB** allele, and the O37 strain had an El Tor CTXphi (rstR(ET) allele) and novel ctxAB alleles. Although the O53 and O65 strains lacked the ctxAB genes, they carried a pre-CTXphi (i.e., rstR(cla)). Identification of non-O1 and non-O139 serogroups with pathogenic potential in epidemic genetic backgrounds means that attention should be paid to possible future epidemics caused by these serogroups and to the need for new, rapid vaccine development strategies.

L20 ANSWER 5 OF 128 MEDLINE DUPLICATE 4

2002119833 Document Number: 21843114. PubMed ID: 11854209. Mutational analysis of ganglioside GM(1)-binding ability, pentamer formation, and epitopes of cholera toxin B (CTB) subunits and CTB/heat-labile enterotoxin B subunit chimeras. Jobling Michael G; Holmes Randall K. (Department of Microbiology, University of Colorado Health Sciences Center, Denver, Colorado 80262, USA.) INFECTION AND IMMUNITY, (2002 Mar) 70 (3) 1260-71. Journal code: 0246127. ISSN: 0019-9567. Pub. country: United States. Language: English.

AB Variants of cholera toxin B subunit (CTB) were made by bisulfite- and oligonucleotide-directed mutagenesis of the **ctxB** gene. Variants were screened by a radial passive immune hemolysis assay (RPIHA) for loss of binding to sheep erythrocytes (SRBC). Variant CTBs were characterized for the formation of immunoreactive pentamers, the ability to bind ganglioside GM(1) in vitro, and reactivity with a panel of monoclonal anti-CTB antibodies. Substitutions at eight positions (i.e., positions 22, 29, 36, 45, 64, 86, 93, and 100) greatly reduced the yield of

immunoreactive CTB. RPIHA-negative substitution variants that formed immunoreactive pentamers were obtained for residues 12, 33, 36, 51, 52 + 54, 91, and 95. Tyrosine-12 was identified as a novel residue important for GM(1) binding since, among all of the novel variants isolated with altered RPIHA phenotypes, only CTB with aspartate substituted for tyrosine at position 12 failed to bind significantly to ganglioside GM(1) in vitro. In contrast, CTB variants with single substitutions for several other residues (Glu-51, Lys-91, and Ala-95) that participate in GM(1) binding, based on the crystal structure of CTB and the oligosaccharide of GM(1), were not appreciably altered in their ability to bind GM(1) in vitro, even though they showed altered RPIHA phenotypes and did not bind to SRBC. Hybrid B genes made by fusing **ctxB** and the related Escherichia coli heat-labile enterotoxin eltB genes at codon 56 produced CTB variants that had 7 or 12 heat-labile enterotoxin B residue substitutions in the amino or carboxyl halves of the monomer, respectively, each of which which also bound GM(1) as well as wild-type CTB. This collection of variant CTBs in which 47 of the 103 residues were substituted was used to map the epitopes of nine anti-CTB monoclonal antibodies (MAbs). Each MAb had a unique pattern of reactivity with the panel of CTB variants. Although no two of the epitopes recognized by different MAbs were identical, most of the single amino acid substitutions that altered the immunoreactivity of CTB affected more that one epitope. The tertiary structures of the epitopes of these anti-CTB MAbs are highly conformational and may involve structural elements both within and between CTB monomers. Substitution of valine for alanine at positions 10 and 46 had dramatic effects on the immunoreactivity of CTB, affecting epitopes recognized by eight or six MAbs, respectively.

L20 ANSWER 6 OF 128 MEDLINE DUPLICATE 5

2002150343 Document Number: 21877323. PubMed ID: 11882700. Contribution of the ADP-ribosylating and receptor-binding properties of cholera-like enterotoxins in modulating cytokine secretion by human intestinal epithelial cells. Soriani Marco; Bailey Lorna; Hirst Timothy R. (Department of Pathology and Microbiology, University of Bristol, Bristol BS8 1TD, UK.) MICROBIOLOGY, (2002 Mar) 148 (Pt 3) 667-76. Journal code: 9430468. ISSN: 1350-0872. Pub. country: England: United Kingdom. Language: English.

AB When epithelial cells first encounter cholera toxin (Ctx) produced by Vibrio cholerae they secrete not only chloride ions responsible for causing diarrhoea, but also a number of cytokines that may contribute to the toxin's potent immunomodulatory properties. Much less is known about the ability of the heat-labile enterotoxin of Escherichia coli (Etx), a close homologue of Ctx, to elicit cytokine secretion by epithelial cells. This study shows that treatment of human intestinal epithelial T84 cells with Etx induces expression of IL-6, IL-10, IL-1R antagonist, as well as IL-1alpha and IL-1beta and low levels of IL-8. Such induction was totally dependent on the intrinsic ADP-ribosylating activity of the toxin A-subunit, and could be mimicked by cAMP-elevating agents, such as forskolin and dibutyryl cAMP. By comparison, neither an enzymically inactive mutant of Etx nor EtxB was able to induce cytokine secretion. The behaviour of Ctx and **CtxB** was very similar to that of Etx and EtxB, respectively. The spectrum of cytokines released by Etx and Ctx indicates that the toxins may create a local microenvironment that strongly biases the immune response towards an anti-inflammatory and a polarized Th2 response.

L20 ANSWER 7 OF 128 MEDLINE DUPLICATE 6

2002249411 Document Number: 21984494. PubMed ID: 11951093. A distinct class of endosome mediates clathrin-independent endocytosis to the Golgi complex. Nichols B J. (MRC Laboratory of Molecular Biology, Hills Road, Cambridge CB2 2QH, UK.) NATURE CELL BIOLOGY, (2002 May) 4 (5) 374-8. Journal code: 100890575. ISSN: 1465-7392. Pub. country: England: United Kingdom. Language: English.

AB Mammalian cells endocytose a variety of proteins and lipids without utilising clathrin-coated pits. Detailed molecular mechanisms for clathrin-independent endocytosis are unclear. Several markers for this process, including glycosphingolipid-binding bacterial toxin subunits such as cholera toxin B subunit (**CTxB**), and glycosyl-phosphatidyl-inositol (GPI)-anchored proteins, are found in detergent-resistant membrane fractions (DRMs), or 'lipid rafts'. The Golgi complex constitutes one principal intracellular destination for these markers. Uptake of both **CTxB** and GPI-anchored proteins may involve caveolae, small invaginations in the plasma membrane (PM). However, the identity of intermediate organelles involved in PM to Golgi trafficking, as well as the function of caveolins, defining protein components of caveolae, are unclear. This paper shows that molecules which partition into DRMs and are endocytosed in a clathrin-independent fashion, accumulate in a discrete population of endosomes en route to the Golgi complex. These endosomes are devoid of markers for classical early and recycling endosomes, but do contain caveolin-1. Caveolin-1-positive endosomes are sites for the sorting of caveolin-1 away from Golgi-bound cargoes, although caveolin-1 itself is unlikely to have a direct function in PM to Golgi transport.

L20 ANSWER 8 OF 128 SCISEARCH COPYRIGHT 2002 ISI (R) DUPLICATE 7
2002:388868 The Genuine Article (R) Number: 546JR. Construction of a trivalent candidate vaccine against Shigella species with DNA recombination. Wang H L (Reprint); Feng E L; Lin Y; Liao X; Jin M; Huang L Y; Su G F; Huang C F. Beijing Inst Biotechnol, Beijing 100071, Peoples R China (Reprint). SCIENCE IN CHINA SERIES C-LIFE SCIENCES (FEB 2002) Vol. 45, No. 1, pp. 10-20. Publisher: SCIENCE PRESS. 16 DONGHUANGCHENGGEN NORTH ST, BEIJING 100717, PEOPLES R CHINA. ISSN: 1006-9305. Pub. country: Peoples R China. Language: English.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS
AB In this work asd gene of Shigella flexneri 2a strain T32 was replaced by Vibrio cholerae toxin B subunit (**ctxB**) gene with DNA recombination in vivo and in vitro. The resulting derivative of T32, designed as FWL01, could stably express **CtxB**, but its growth in LB medium depended on the presence of diaminopimelic acid (DAP). Then form I plasmid of Shigella sonnei strain S7 was labeled with strain T32 asd gene and mobilized into FWL01. Thus a trivalent candidate oral vaccine strain, designed as FSWO1, was constructed. In this candidate strain, a balanced-lethal system was constituted between the host strain and the form I plasmid expressing S. sonnei O antigen. Therefore the candidate strain can express stably not only its own O antigen but also **CtxB** and 0 antigen of S. sonnei in the absence of any antibiotic. Experiments showed that FSW01 did not invade HeLa cells or cause keratoconjunctivitis in guinea pigs. However, rabbits immunized FSW01 can elicit significant immune responses. In mice and rhesus monkey models, vaccinated animals were protected against the challenges of wild S. flexneri 2a strain 2457T and S. sonnei strain S9.

L20 ANSWER 9 OF 128 CAPLUS COPYRIGHT 2002 ACS
2001:693488 Document No. 135:252774 Genetically stable mutant strains of Vibrio cholerae lacking functional flagellum, and uses thereof as vaccines. Hase, Claudia; Mekalanos, John J. (St. Jude Children's Research Hospital, USA; President and Fellows of Harvard College). PCT Int. Appl. WO 2001068829 A2 20010920, 105 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2001-US8305 20010316. PRIORITY: US 2000-526344 20000316.

AB The invention relates to nontoxigenic genetically stable mutant strains of Vibrio cholerae which lack functional flagellum and are useful as a vaccine for inducing immunol. protection against cholera and a method for making the same. The strains of the present invention comprise a genetically engineered deletion mutation resulting in loss of at least part of a gene encoding a protein required for the energization or assembly of flagellum. In particular, mutant strains lacking one or more functional genes selected from the group of motX, motY, pomA and pomA/pomB are provided. In addn. mutant strains lacking a functional hap gene and/or ctxA gene and/or rtx gene, in combination with one or more of the motX, motY, pomA and pomA/pomB and fliG genes are provided. Mutant strains further comprising alterations wherein expression of the **ctxB** gene can be induced are also provided. The invention further provides methods and compns. for cholera vaccines comprising the disclosed mutant strains.

L20 ANSWER 10 OF 128 MEDLINE DUPLICATE 8
2001419634 Document Number: 21353030. PubMed ID: 11447291. A mutant cholera toxin B subunit that binds GM1- ganglioside but lacks immunomodulatory or toxic activity. Aman A T; Fraser S; Merritt E A; Rodigherio C; Kenny M; Ahn M; Hol W G; Williams N A; Lencer W I; Hirst T R. (Department of Pathology and Microbiology, University of Bristol, Bristol BS81TD, United Kingdom.) PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (2001 Jul 17) 98 (15) 8536-41. Journal code: 7505876. ISSN: 0027-8424. Pub. country: United States. Language: English.

AB GM1-ganglioside receptor binding by the B subunit of cholera toxin (**CtxB**) is widely accepted to initiate toxin action by triggering uptake and delivery of the toxin A subunit into cells. More recently, GM1 binding by isolated **CtxB**, or the related B subunit of Escherichia coli heat-labile enterotoxin (EtxB), has been found to modulate leukocyte function, resulting in the down-regulation of proinflammatory immune responses that cause autoimmune disorders such as rheumatoid arthritis and diabetes. Here, we demonstrate that GM1 binding, contrary to expectation, is not sufficient to initiate toxin action. We report the engineering and crystallographic structure of a mutant cholera toxin, with a His to Ala substitution in the B subunit at position 57. Whereas the mutant retained pentameric stability and high affinity binding to GM1-ganglioside, it had lost its immunomodulatory activity and, when part of the holotoxin complex, exhibited ablated toxicity. The implications of these findings on the mode of action of cholera toxin are discussed.

L20 ANSWER 11 OF 128 MEDLINE DUPLICATE 9
2001551428 Document Number: 21481952. PubMed ID: 11598049. Outer membrane targeting of passenger proteins by the vacuolating cytotoxin autotransporter of Helicobacter pylori. Fischer W; Buhrdorf R; Gerland E; Haas R. (Max von Pettenkofer-Institut fur Hygiene und Medizinische Mikrobiologie, Ludwig-Maximilians-Universitat, Munich, Germany.. schmitt@m3401.mpk.med.uni-muenchen.de) . INFECTION AND IMMUNITY, (2001 Nov) 69 (11) 6769-75. Journal code: 0246127. ISSN: 0019-9567. Pub. country: United States. Language: English.

AB Helicobacter pylori produces a number of proteins associated with the outer membrane, including adhesins and the vacuolating cytotoxin. These proteins are supposed to integrate into the outer membrane by beta-barrel structures, characteristic of the family of autotransporter proteins. By using the SOMPES (shuttle vector-based outer membrane protein expression) system for outer membrane protein production, we were able to functionally express in H. pylori the cholera toxin B subunit genetically fused to the C-terminal VacA domain. We demonstrate that the fusion protein is translocated to the H. pylori outer membrane and that the **CtxB** domain is exposed on the H. pylori surface. Thus, we provide the first experimental evidence that the C-terminal beta-domain of VacA can

transport a foreign passenger protein to the H. pylori surface and hence acts as a functional autotransporter.

L20 ANSWER 12 OF 128 MEDLINE DUPLICATE 10
2001248162 Document Number: 21189275. PubMed ID: 11292779. Escherichia coli heat-labile enterotoxin B subunit is a more potent mucosal adjuvant than its vlosely related homologue, the B subunit of cholera toxin. Millar D G; Hirst T R; Snider D P. (Department of Pathology and Molecular Medicine, McMaster University, Hamilton, Ontario, Canada L8N 3Z5.. dmillar@uhnres.utoronto.ca) . INFECTION AND IMMUNITY, (2001 May) 69 (5) 3476-82. Journal code: 0246127. ISSN: 0019-9567. Pub. country: United States. Language: English.

AB Although cholera toxin (Ctx) and Escherichia coli heat-labile enterotoxin (Etx) are known to be potent mucosal adjuvants, it remains controversial whether the adjuvanticity of the holotoxins extends to their nontoxic, receptor-binding B subunits. Here, we have systematically evaluated the comparative adjuvant properties of highly purified recombinant EtxB and **CtxB**. EtxB was found to be a more potent adjuvant than **CtxB**, stimulating responses to hen egg lysozyme when the two were coadministered to mice intranasally, as assessed by enhanced serum and secretory antibody titers as well as by stimulation of lymphocyte proliferation in spleen and draining lymph nodes. These results indicate that, although structurally very similar, EtxB and **CtxB** have strikingly different immunostimulatory properties and should not be considered equivalent as prospective vaccine adjuvants.

L20 ANSWER 13 OF 128 MEDLINE DUPLICATE 11
2001192726 Document Number: 21105201. PubMed ID: 11160664. Protective mucosal immunity to ocular herpes simplex virus type 1 infection in mice by using Escherichia coli heat-labile enterotoxin B subunit as an adjuvant. Richards C M; Aman A T; Hirst T R; Hill T J; Williams N A. (Department of Pathology and Microbiology, School of Medical Sciences, University of Bristol, Bristol BS8 1TD, United Kingdom.. Claire.M.Richards@bristol.ac.uk) . JOURNAL OF VIROLOGY, (2001 Feb) 75 (4) 1664-71. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB The potential of nontoxic recombinant B subunits of cholera toxin (rCtxB) and its close relative Escherichia coli heat-labile enterotoxin (rEtxB) to act as mucosal adjuvants for intranasal immunization with herpes simplex virus type 1 (HSV-1) glycoproteins was assessed. Doses of 10 microg of rEtxB or above with 10 microg of HSV-1 glycoproteins elicited high serum and mucosal anti-HSV-1 titers comparable with that obtained using **CtxB** (10 microg) with a trace (0.5 microg) of whole toxin (Ctx-**CtxB**). By contrast, doses of rCtxB up to 100 microg elicited only meager anti-HSV-1 responses. As for Ctx-**CtxB**, rEtxB resulted in a Th2-biased immune response with high immunoglobulin G1 (IgG1)/IgG2a antibody ratios and production of interleukin 4 (IL-4) and IL-10 as well as gamma interferon by proliferating T cells. The protective efficacy of the immune response induced using rEtxB as an adjuvant was assessed following ocular challenge of immunized and mock-immunized mice. Epithelial disease was observed in both groups, but the immunized mice recovered by day 6 whereas mock-immunized mice developed more severe corneal disease leading to stromal keratitis. In addition, a significant reduction in the incidence of lid disease and zosteriform spread was observed in immunized animals and there was no encephalitis compared with 95% encephalitis in mock-immunized mice. The potential of such mucosal adjuvants for use in human vaccines against pathogens such as HSV-1 is discussed.

L20 ANSWER 14 OF 128 CAPLUS COPYRIGHT 2002 ACS
2002:97454 Document No. 137:2910 Study on several toxigenes detection of Vibrio cholerae. Zhang, Zheng; Zhu, Shuirong; Huang, Liang; Chen, Xiuying; Mo, Shuntang (Zhejiang Provincial Center for Disease Prevention

and Control, Hangzhou, 310009, Peop. Rep. China). Zhongguo Gonggong Weisheng, 17(12), 1095-1096 (Chinese) 2001. CODEN: ZGWEE3. ISSN: 1001-0580. Publisher: Zhongguo Gonggong Weisheng Zazhishe.

AB The pos. rates of several main toxigenes in the strains of Vibrio cholerae isolated in Zhejiang Province were studied. Ninety-one strains of vibrio cholerae isolated from Zhejiang Province for toxigene were detected with toxigene probes labeled with Digoxigenin. Toxigenes such as ctxAB, Zot, and Ace generally existed in epidemic strains of EVC and VC O139, pos. rate of these three genes was 100, 98.86, and 96.59%, resp., and these three genes did not exist in non-epidemic strains of EVC. It was showed that more attention should be paid to surveillance and study of epidemic EVC and VC O139. Restriction fragment length polymorphism hybridization anal. of ctxAB gene indicated that VC O139 was similar to epidemic EVC in hereditary characteristics, and there were two copies in both of them.

L20 ANSWER 15 OF 128 MEDLINE DUPLICATE 12

2001446632 Document Number: 21385422. PubMed ID: 11494170. Local production of anti-vibrio cholerae mucosal antibody in reproductive tract tissues after cholera. Ryan E T; Bridges E A; Crean T I; Gausia K; Hamadani J D; Aziz A; Hawkes S; Begum M; Bogaerts J; Faruque S M; Salam M A; Fuchs G J; Calderwood S B. (Tropical and Geographic Medicine Center, Division of Infectious Diseases, Massachusetts General Hospital, Boston, MA 02114, USA.. etryan@partners.org) . JOURNAL OF INFECTIOUS DISEASES, (2001 Sep 1) 184 (5) 643-7. Journal code: 0413675. ISSN: 0022-1899. Pub. country: United States. Language: English.

AB To investigate whether intestinal presentation of an antigen by Vibrio cholerae, a noninvasive organism, could induce an anatomically distant mucosal immune response in reproductive tract tissues, the endocervical immune responses of women in Bangladesh were evaluated after cholera. Endocervical secretions were analyzed for secretory IgA (sIgA) antibody against the B subunit of cholera toxin (**CtxB**) in 9 women with cholera and 8 women with diarrhea caused by neither V. cholerae nor heat labile enterotoxin-producing Escherichia coli. Women infected with V. cholerae developed significant sIgA anti-**CtxB** responses in endocervical samples (P< or =.02). Antibody subtype analysis of endocervical IgA was consistent with local mucosal production (P< or =.001). Women with cholera did not develop sIgA anti-**CtxB** responses in serum. The ability to generate specific mucosal immune responses in reproductive tract tissues after intestinal presentation of antigen could facilitate development of vaccines effective against reproductive tract pathogens.

L20 ANSWER 16 OF 128 SCISEARCH COPYRIGHT 2002 ISI (R)

2001:320246 The Genuine Article (R) Number: 421KA. Evidence for a role of ganglioside GM(1) in antigen presentation: binding enhances presentation of Escherichia coli enterotoxin B subunit (EtxB) to CD4(+) T cells. Nashar T O (Reprint); Betteridge Z E; Mitchell R N. Univ Bristol, Sch Med Sci, Dept Pathol & Microbiol, Bristol BS8 1TD, Avon, England (Reprint); Brigham & Womens Hosp, Dept Pathol, Boston, MA 02115 USA; Harvard Univ, Sch Med, Boston, MA 02115 USA. INTERNATIONAL IMMUNOLOGY (APR 2001) Vol. 13, No. 4, pp. 541-551. Publisher: OXFORD UNIV PRESS. GREAT CLARENDON ST, OXFORD OX2 6DP, ENGLAND. ISSN: 0953-8178. Pub. country: England; USA. Language: English.

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Successful antigen presentation by antigen-presenting cells is governed by a number of factors including the efficiency of antigen capture by cell-surface receptors, targeting to compartments of antigen processing, surface expression of MHC II-peptide complexes and presence of costimulatory signals. Ganglioside GM(1) is an important component of membrane glycosphingolipids, and has been implicated in cell differentiation, apoptosis and signal transduction pathways. Using the a subunit of Escherichia coll enterotoxin (EtxB), a potent immunogen that binds GM1 with high affinity, and a non-binding mutant of EtxB,

EtxB((G33D)), we demonstrate that GM(1) is intimately involved in several aspects of antigen presentation. Thus, GM(1)-mediated presentation of EtxB by a cells and CD11c(+) dendritic cells (DC) significantly enhanced the proliferation and cytokine expression of EtxB-specific CD4(+) T cells. Investigation regarding potential mechanisms revealed that EtxB binding directly augments the expression of MHC class H on B cells, and fractionation of a cells demonstrated that EtxB binding to GM(1) results in rapid internalization and targeting to class II-rich compartments. GM(1)-mediated uptake of antigens and access to class II compartments in B cells can be exploited to significantly enhance the presentation of ovalbumin-conjugated to EtxB. These results demonstrate that GM(1) can play an important role in antigen presentation via the MHC II pathway.

L20 ANSWER 17 OF 128 MEDLINE DUPLICATE 13

2001309548 Document Number: 21229701. PubMed ID: 11331304. Rapid cycling of lipid raft markers between the cell surface and Golgi complex. Nichols B J; Kenworthy A K; Polishchuk R S; Lodge R; Roberts T H; Hirschberg K; Phair R D; Lippincott-Schwartz J. (Cell Biology and Metabolism Branch, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, Maryland 20895, USA.) JOURNAL OF CELL BIOLOGY, (2001 Apr 30) 153 (3) 529-41. Journal code: 0375356. ISSN: 0021-9525. Pub. country: United States. Language: English.

AB The endocytic itineraries of lipid raft markers, such as glycosyl phosphatidylinositol (GPI)-anchored proteins and glycosphingolipids, are incompletely understood. Here we show that different GPI-anchored proteins have different intracellular distributions; some (such as the folate receptor) accumulate in transferrin-containing compartments, others (such as CD59 and GPI-linked green fluorescent protein [GFP]) accumulate in the Golgi apparatus. Selective photobleaching shows that the Golgi pool of both GPI-GFP and CD59-GFP constantly and rapidly exchanges with the pool of these proteins found on the plasma membrane (PM). We visualized intermediates carrying GPI-GFP from the Golgi apparatus to the PM and separate structures delivering GPI-GFP to the Golgi apparatus.GPI-GFP does not accumulate within endocytic compartments containing transferrin, although it is detected in intracellular structures which are endosomes by the criteria of accessibility to a fluid phase marker and to cholera and shiga toxin B subunits (**CTxB** and STxB, which are also found in rafts). GPI-GFP and a proportion of the total **CTxB** and STxB taken up into cells are endocytosed independently of clathrin-associated machinery and are delivered to the Golgi complex via indistinguishable mechanisms. Hence, they enter the Golgi complex in the same intermediates, get there independently of both clathrin and rab5 function, and are excluded from it at 20 degrees C and under conditions of cholesterol sequestration. The PM-Golgi cycling pathway followed by GPI-GFP could serve to regulate lipid raft distribution and function within cells.

L20 ANSWER 18 OF 128 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.

2002128850 EMBASE Rapid cycling of lipid raft markers between the cell surface and golgi complex. Nichols B.J.; Kenworthy A.K.; Polishchuk R.S.; Lodge R.; Roberts T.H.; Hirschberg K.; Phair R.D.; Lippincott-Schwartz J.. J. Lippincott-Schwartz, Cell Biology and Metabolism Branch, Natl. Inst. Child Hlth./Human Devmt., Building 18T, Library Drive, Bethesda, MD 20895-0001, United States. jlippin@helix.nih.gov. Journal of Cell Biology 152/3 (529-541) 5 Feb 2001.
Refs: 39.
ISSN: 0021-9525. CODEN: JCLBA3. Pub. Country: United States. Language: English. Summary Language: English.

AB The endocytic itineraries of lipid raft markers, such as glycosyl phosphatidylinositol (GPI)anchored proteins and glycosphingolipids, are incompletely understood. Here we show that different GPI-anchored proteins have different intracellular distributions; some (such as the folate receptor) accumulate in transferrin-containing compartments, others (such as CD59 and GPI-linked green fluorescent protein [GFP]) accumulate in the

Golgi apparatus. Selective photobleaching shows that the Golgi pool of both GPI-GFP and CD59-GFP constantly and rapidly exchanges with the pool of these proteins found on the plasma membrane (PM). We visualized intermediates carrying GPI-GFP from the Golgi apparatus to the PM and separate structures delivering GPI-GFP to the Golgi apparatus. GPI-GFP does not accumulate within endocytic compartments containing transferrin, although it is detected in intracellular structures which are endosomes by the criteria of accessibility to a fluid phase marker and to cholera and shiga toxin B subunits (**CTxB** and STxB, which are also found in rafts). GPI-GFP and a proportion of the total **CTxB** and STxB taken up into cells are endocytosed independently of clathrin-associated machinery and are delivered to the Golgi complex via indistinguishable mechanisms. Hence, they enter the Golgi complex in the same intermediates, get there independently of both clathrin and rab5 function, and are excluded from it at 20.degree.C and under conditions of cholesterol sequestration. The PM-Golgi cycling pathway followed by GPI-GFP could serve to regulate lipid raft distribution and function within cells.

L20 ANSWER 19 OF 128 CAPLUS COPYRIGHT 2002 ACS

2001:825686 Construction of the **CtxB**-expressing vector with the non-antibiotic gene marker. Wang, Hengliang; Feng, Erling; Liu, Mei; Liao, Xiang; Shi, Zhaoxing; Yao, Xiao; Huang, Liuyu; Su, Guofu (Beijing Institute of Biotechnology, Academy of Military Medical Sciences, Beijing, 100071, Peop. Rep. China). Xibao Yu Fenzi Mianyixue Zazhi, 17(5), 428-429 (Chinese) 2001. CODEN: XFMZFM. ISSN: 1007-8738. Publisher: Disi Junyi Daxue.

AB A **CtxB**-expressing vector carrying a non-antibiotic gene marker was constructed. The mercuric-resistance gene with a length of 2.9 kb was obtained from plasmid pMT999 by PCR. It was ligated to origin of replication of pBR322, resulting the recombinant plasmid pBRH02. Then the expression vector was constructed by inserting the **CtxB** gene with .beta.-lactamase promoter into pBRH02. After pRBC09 was transformed into E.coli and Shigella spp., the effective expression of **CtxB** in them was detected by GM1-ELISA. Using the mercuric resistance gene and origin of replication of pBR322, the recombinant plasmid, which expresses **CtxB** and carries a non-antibiotic gene marker, was successfully constructed.

L20 ANSWER 20 OF 128 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
DUPLICATE 14

2001:290633 Document No.: PREV200100290633. A candidate oral vaccine to Helicobacter pylori: Fusion protein of HspA and **CtxB**. Li Ming-Feng; He Zhi-Yong; Ling Zheng; Wang Jiao-Yang; Sheng Xu-Dong; Yang Guan-Zhen; Wu Xiang-Fu (1). (1) Shanghai Institute of Biochemistry, Chinese Academy of Sciences, Shanghai, 200031: xfwu@sunm.shcnc.ac.cn China. Shengwu Huaxue yu Shengwu Wuli Xuebao, (May, 2001) Vol. 33, No. 3, pp. 360-364. print. ISSN: 0582-9879. Language: Chinese. Summary Language: Chinese; English.

AB Heat-shock protein A subunit (HspA), an effective immunogen may stimulate the immunoresponse in human body against challenge of H. pylori. The B subunit of cholera toxin (**CtxB**) has been proved to be a potent mucosal immunogen, act as an adjuvant for vaccine targeted for delivery to the mucosal-associated lymphoid tissue. A recombinant plasmid expressing bivalent antigen of HspA and **CtxB** subunit was constructed as follows. hspA and **ctxB** gene was amplified by PCR. The DNA products of hspA and **ctxB** were inserted in the prokaryotic expression vector pET-22b(+), respectively, and then the resulted recombinant plasmid expressing a fusion protein named HCT was transformed into the E. coli strain BL-21(DE3). hct gene was measured to be 708 base pairs long, and the fusion protein encoding a polypeptide of 236 amino acid residues, corresponded to a calculated molecular masses of 30 kD. Western blot analysis of the recombinant protein HCT confirmed that it could be specifically recognized by the serum of H. pylori-infected

patients. HspA and HCT labelled 125I were orally administered into the stomach of mice, respectively, and the radioactivity of 125I in serum of each mouse was assayed at intervals: 15 min, 30 min, 60 min, 90 min and 120 min. The result indicated that there were high radioactivity counts in the groups of HCT than that of HspA ($P < 0.001$). This result suggests that the **CtxB** may enhance the volume of HspA absorbed from the intestine of mice, therefore the recombinant fusion protein HCT may be an effective oral vaccine for prevention and treatment against the infection of H. pylori.

L20 ANSWER 21 OF 128 MEDLINE DUPLICATE 15

2001210820 Document Number: 21196418. PubMed ID: 11298654. Cholera toxin and Escherichia coli enterotoxin B-subunits inhibit macrophage-mediated antigen processing and presentation: evidence for antigen persistence in non-acidic recycling endosomal compartments. Millar D G; Hirst T R. (Department of Pathology and Microbiology, University of Bristol, School of Medical Sciences, Bristol BS8 1TD, UK.) CELLULAR MICROBIOLOGY, (2001 May) 3 (5) 311-29. Journal code: 100883691. ISSN: 1462-5814. Pub. country: England: United Kingdom. Language: English.

AB Cholera toxin (Ctx) and the closely related Escherichia coli heat-labile enterotoxin (Etx) not only act as mediators of diarrhoeal disease but also exert potent immunomodulatory properties on mammalian immune systems. The toxins normally exert their diarrhoeagenic effects by initiating receptor-mediated uptake into vesicles that enter a retrograde trafficking pathway, circumventing degradative compartments and targeting them to the trans-Golgi network (TGN) and endoplasmic reticulum. Here, we examine whether receptor-mediated binding and cellular entry by the toxin B-subunits also lead to concomitant changes in uptake and trafficking of exogenous antigens that could contribute to the potent immunomodulatory properties of these toxins. Treatment of the macrophage (J774.2) cell line with Etx B-subunit (EtxB) resulted in EtxB transport to the TGN and also led to the formation of large, translucent, non-acidic, EtxB-devoid vacuoles. When exogenous antigens were added, EtxB-treated cells were found to be proficient in both internalization of ovalbumin (OVA) and phagocytosis of bacterial particles. However, the internalized OVA, instead of trafficking along a lysosome-directed endocytic pathway via acidified endosomes, persisted in a non-acidic, light-density compartment that was distinct from the translucent vacuoles. The rerouted OVA did not co-localize with the endosomal markers rab5 or rab11, nor with EtxB, but was retained in a transferrin receptor-positive compartment. The failure of OVA to enter the late endosomal/lysosomal compartments correlated with a striking inhibition of OVA peptide processing and presentation to OVA-responsive CD4+ T-cells. **CtxB** also modulated OVA trafficking and inhibited antigen presentation. These findings demonstrate that the B-subunits of Ctx and Etx alter the progression of exogenous antigens along the endocytic processing pathway, and prevent or delay efficient epitope presentation and T-cell stimulation. The formation of such 'antigen depots' could contribute to the immunomodulatory properties of these bacterial virulence determinants.

L20 ANSWER 22 OF 128 MEDLINE DUPLICATE 16

2001206192 Document Number: 21124873. PubMed ID: 11222115. Peroral immunization with Helicobacter pylori adhesin protein genetically linked to cholera toxin A2B subunits. Kim B O; Shin S S; Yoo Y H; Pyo S. (School of Pharmacy, Sung Kyun Kwan University, Suwon, 440-746, Kyunggi-Do, South Korea.) CLINICAL SCIENCE, (2001 Mar) 100 (3) 291-8. Journal code: 7905731. ISSN: 0143-5221. Pub. country: England: United Kingdom. Language: English.

AB Helicobacter pylori is a major cause of gastric-associated diseases. To evaluate the efficacy of a possible vaccine antigen against H. pylori infection, the chimaeric construct adhesin--CTXA2B, derived from H. pylori adhesin genetically coupled to cholera toxin (CTX) subunits A2 and B (CTXA2B), was expressed in Escherichia coli as an insoluble recombinant

chimaeric protein. The protein was then purified by denaturation, renaturation and size-exclusion chromatography. The composition of purified adhesin--CTXA2B was verified by SDS/PAGE and Western blotting with antibodies to antigenic components of adhesin and **CTXB**, and confirmed as a chimaeric protein with G(M1)-ganglioside binding activity and adhesin epitopes by a G(M1)-ELISA developed using antibodies to adhesin. Oral immunization of mice with adhesin--CTXA2B induced higher levels of mucosal IgA and serum IgG antibodies to H. pylori adhesin and to **CTXB** than in mice immunized with adhesin or CTXA2B alone. Adhesin--CTXA2B was also demonstrated to be a potential protective antigen in a mouse model of H. pylori infection. The immunization of mice with adhesin--CTXA2B protected 62.5% of mice infected with H. pylori SS1 strain, whereas adhesin immunization was not able to confer protection to mice. This protection may be correlated with high levels of mucosal IgA and serum IgG antibodies against H. pylori adhesin. Taken together, the results indicate that the genetically linked CTXA2B acts as a useful mucosal adjuvant, and that the adhesin-CTXA2B chimaeric protein could be a potential component in future H. pylori vaccine development.

L20 ANSWER 23 OF 128 MEDLINE DUPLICATE 17
2001152074 Document Number: 21122435. PubMed ID: 11232774. Molecular characterisation of rough variants of Vibrio cholerae isolated from hospitalised patients with diarrhoea. Mitra R K; Nandy R K; Ramamurthy T; Bhattacharya S K; Yamasaki S; Shimada T; Takeda Y; Nair G B. (National Institute of Cholera and Enteric Diseases, Beliaghata, Calcutta, India.) JOURNAL OF MEDICAL MICROBIOLOGY, (2001 Mar) 50 (3) 268-76. Journal code: 0224131. ISSN: 0022-2615. Pub. country: England: United Kingdom. Language: English.

AB Seven rough isolates of Vibrio cholerae isolated as the sole infecting agent from patients with cholera-like diarrhoea were examined for the presence of the regulatory element toxR and certain virulence-associated genes of the CTX genetic element and V. cholerae pathogenicity island (VPI). Multiplex PCR analysis with wb-specific genes of either O1 or O139 origin showed that six of the seven isolates produced an O1 wb-specific amplicon and the remaining isolate produced an O139-specific amplicon. Analysis of lipopolysaccharide profiles of smooth variants of V. cholerae revealed the presence of long repeated units of 'O' polysaccharide side chains but all the rough variants appeared to be devoid of the latter and possessed only core oligosaccharide. PCR amplification with primers specific to the ctxA, **ctxB**, tcpA, tagA, int, aldA, toxT, LJ, RJ and toxR genes revealed that six of the seven rough isolates were positive for these genes. One isolate was found to be negative for tagA and RJ, indicating the presence of an altered VPI. Each of these isolates showed media-dependent expression of cholera toxin (CT) and produced more toxin than the reference V. cholerae O1 El Tor strain VC20 or O139 strain SG24 under comparable conditions. Studies on the clonality of these isolates by the analysis of rRNA genes indicated their relatedness to strains of V. cholerae O1 El Tor or O139, isolated during the same time period.

L20 ANSWER 24 OF 128 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.DUPLICATE 18
2002064630 EMBASE Molecular epidemiology of newly emerged V. cholerae O139 in Taiwan. Lin C.-S.; Wang T.-K.; Lee C.-L.; Pan T.-M.; Tsai J.-L.; Ho S.-I.; Lu C.-H.. C.-L. Lee, Bacteriology Division, Center of Disease Control, Department of Health, 161 Kuen Yang Street, Nangkang 115 Taipei, Taiwan, China. chihli@cdc.gov.tw. Journal of Food and Drug Analysis 9/4 (224-231) 2001.
Refs: 41.
ISSN: 1021-9498. CODEN: YSFEEP. Pub. Country: Taiwan,Province of China. Language: English. Summary Language: English.

AB A Vibrio cholerae O139 strain was isolated from a cholera patient in August 1997 in Kaohsiung County, Taiwan. This was the first case of V. cholerae O139 emerging in Taiwan. An epidemiological study showed that the infectious source was turtle eggs. One clinical isolate and fourteen

isolates from environmental specimens of the turtle farm were collected. From all isolates, the genes encoded for cholera toxin (ctxA and **ctxB**) and for toxin-coregulated pili genes (tcpA and tcpI) were specifically amplified by polymerase chain reactions. In pulsed-field gel electrophoresis (PFGE) studies, these isolates were categorized to five subtypes using SfiI restriction digestion: whereas three distinct subtypes were identified when NotI was used for digestion. The banding pattern of the clinical isolate in PFGE only differed by 1-3 bands from those of the environmental isolates regardless of the restriction enzyme used. The Dice coefficients were found to be in the range of 0.88-1.0 and 0.87-1.0, respectively, by using SfiI and NotI for the subtyping. When these isolates were analyzed by plasmid profile analysis, the profile pattern of the clinical strain was identical to 12 of the 14 environmental strains. Based on these results, it was concluded that the fifteen strains studied were descended from the same origin, with the clinical isolate originated from the environmental isolates. The data suggests that the molecular subtyping is a powerful tool for tracing and verifying the infectious sources of V. cholerae cases.

L20 ANSWER 25 OF 128 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
2002:176294 Document No.: PREV200200176294. Prevalence of virulence-associated genes in clinical and environmental Vibrio cholerae strains isolated in Brazil between 1991-1999. Vital-Brazil, J. M. (1); Karaolis, D. K. R.; Rodrigues, D. P. (1); Campos, L. C. (1). (1) FIOCRUZ, Rio de Janeiro Brazil. Abstracts of the General Meeting of the American Society for Microbiology, (2001) Vol. 101, pp. 44-45. http://www.asmusa.org/mtgsrc/generalmeeting.htm. print. Meeting Info.: 101st General Meeting of the American Society for Microbiology Orlando, FL, USA May 20-24, 2001 ISSN: 1060-2011. Language: English.

AB Cholera is a life-threatening epidemic disease that occurs worldwide. In January 1991 it re-emerged in many Latin American countries after a period of about 100 years of absence in the continent. In Brazil, cholera accounted for 167,719 documented illnesses and 2,009 deaths between 1991-1999, which occurred mainly in the northeast region (91.9%). However, cholera still remains endemic in most areas of Brazil. V. cholerae includes both pathogenic and nonpathogenic strains that vary in their virulence gene content. A comparative study of the possession of virulence genes in environmental and human V. cholerae strains in Brazil may provide a better understanding of the epidemiology and origin of pathogenic strains. The aim of this study was to determine the prevalence of virulence-associated genes located on the CTX element and V. cholerae pathogenicity island (VPI) among clinical and environmental V. cholerae strains isolated in Brazil. The V. cholerae strains used in this study were isolated between 1991-1999 from 12 Brazilian States and comprised 295 clinical isolates with O1 serogroup and 78 environmental isolates with either O1 or non-O1 antigen. PCR analysis was used for the detection of **ctxB**, zot and ace genes that are located on the CTX element and orf1, tcpA, toxT and int genes which are located on the VPI. Both the CTX element and VPI are essential for epidemic disease. We found that 76.9% of the clinical strains contained all the genes on the CTX element that we assayed while 79.7% of clinical isolates contained all the genes on the VPI we assayed. In addition, 56.4% of the environmental strains possessed **ctxB**, zot and ace genes. Interestingly, we detected isolates that appeared to possess the VPI and lack the CTX element, strains which contained the CTX element and lacked the VPI, and strains which appeared to possess parts of the CTX element and VPI. This study shows that the environment is a potential source for toxigenic V. cholerae strains in Brazil. Our study also demonstrates that there is considerable polymorphism in the genetic structure of the CTX element and VPI between V. cholerae isolates.

L20 ANSWER 26 OF 128 CAPLUS COPYRIGHT 2002 ACS
2000:814337 Document No. 133:361908 Bacteriophage isolated from bacterial

genomes and extrachromosomal elements and methods of use thereof. Karaolis, David K. R. (University of Maryland, Baltimore, USA). PCT Int. Appl. WO 2000067784 A1 20001116, 59 pp. DESIGNATED STATES: W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 2000-US12580 20000510. PRIORITY: US 1999-PV133373 19990510.

AB The present invention relates to compns., methods, processes, etc., relating to bacteriophage which are encoded by chromosome, plasmids, or an extrachromosomal element of bacteria. The bacteriophage of the present invention are preferably encoded by pathogenicity islands in chromosomes or plasmids of pathogenic bacteria. The bacteriophage can be utilized as a pharmaceutical compn., e.g., to elicit an immune response, e.g., for the purpose of producing antibodies, as vaccines and vaccine vectors to regulate the immune system, e.g., for the prevention and treatment of allergy, disease, and other pathol. conditions. The invention finds addnl. utility in systems and methods for the detection of pathogens comprising bacteriophage and a system and method for the environmental eradication of pathogenic microorganisms.

L20 ANSWER 27 OF 128 MEDLINE DUPLICATE 19

2001010966 Document Number: 20444202. PubMed ID: 10986258. Molecular analyses of a putative CTXphi precursor and evidence for independent acquisition of distinct CTX(phi)s by toxigenic Vibrio cholerae. Boyd E F; Heilpern A J; Waldor M K. (Howard Hughes Medical Institute and Division of Geographic Medicine and Infectious Diseases, Tufts-New England Medical Center, Tufts University School of Medicine, Boston, Massachusetts 02111, USA.) JOURNAL OF BACTERIOLOGY, (2000 Oct) 182 (19) 5530-8. Journal code: 2985120R. ISSN: 0021-9193. Pub. country: United States. Language: English.

AB The genes encoding cholera toxin (ctxA and **ctxB**) are encoded in the genome of CTXphi, a filamentous phage that infects Vibrio cholerae. To study the evolutionary history of CTXphi, we examined genome diversity in CTX(phi)s derived from a variety of epidemic and nonepidemic Vibrio sp. natural isolates. Among these were three V. cholerae strains that contained CTX prophage sequences but not the ctxA and **ctxB** genes. These prophages each gave rise to a plasmid form whose genomic organization was very similar to that of the CTXphi replicative form, with the exception of missing ctxAB. Sequence analysis of these three plasmids revealed that they lacked the upstream control region normally found 5' of ctxA, as well as the ctxAB promoter region and coding sequences. These findings are consistent with the hypothesis that a CTXphi precursor that lacked ctxAB simultaneously acquired the toxin genes and their regulatory sequences. To assess the evolutionary relationships among additional CTX(phi)s, two CTXphi-encoded genes, orfU and zot, were sequenced from 13 V. cholerae and 4 V. mimicus isolates. Comparative nucleotide sequence analyses revealed that the CTX(phi)s derived from classical and El Tor V. cholerae isolates comprise two distinct lineages within otherwise nearly identical chromosomal backgrounds (based on mdh sequences). These findings suggest that nontoxigenic precursors of the two V. cholerae O1 biotypes independently acquired distinct CTX(phi)s.

L20 ANSWER 28 OF 128 CAPLUS COPYRIGHT 2002 ACS

2000:642028 Document No. 134:126642 Virulence genes in environmental strains of Vibrio cholerae. Chakraborty, Soumen; Mukhopadhyay, Asish K.; Bhadra, Rupak Kumar; Ghosh, Amar Nath; Mitra, Rupak; Shimada, Toshio; Yamasaki, Shinji; Faruque, Shah M.; Takeda, Yoshifumi; Colwell, Rita R.; Nair, G. Balakrish (National Institute of Cholera and Enteric Diseases, Calcutta, 700 010, India). Applied and Environmental Microbiology, 66(9), 4022-4028

(English) 2000. CODEN: AEMIDF. ISSN: 0099-2240. Publisher: American Society for Microbiology.

AB The virulence of a pathogen is dependent on a discrete set of genetic determinants and their well-regulated expression. The ctxAB and tcpA genes are known to play a cardinal role in maintaining virulence in Vibrio cholerae, and these genes are believed to be exclusively assocd. with clin. strains of O1 and O139 serogroups. In this study, we examd. the presence of five virulence genes, including ctxAB and tcpA, as well as toxR and toxT, which are involved in the regulation of virulence, in environmental strains of V. cholerae cultured from three different freshwater lakes and ponds in the eastern part of Calcutta, India. PCR anal. revealed the presence of these virulence genes or their homologues among diverse serotypes and ribotypes of environmental V. cholerae strains. Sequencing of a part of the tcpA gene carried by an environmental strain showed 97.7% homol. to the tcpA gene of the classical biotype of V. cholerae O1. Strains carrying the tcpA gene expressed the toxin-coregulated pilus (TCP), demonstrated by both autoagglutination anal. and electron microscopy of the TCP pili. Strains carrying ctxAB genes also produced cholera toxin, detd. by mono-sialoganglioside ELISA and by passage in the ileal loops of rabbits. Thus, this study demonstrates the presence and expression of crit. virulence genes or their homologues in diverse environmental strains of V. cholerae, which appear to constitute an environmental reservoir of virulence genes, thereby providing new insights into the ecol. of V. cholerae.

L20 ANSWER 29 OF 128 MEDLINE DUPLICATE 20

2000143721 Document Number: 20143721. PubMed ID: 10678922. In vitro and in vivo analyses of constitutive and in vivo-induced promoters in attenuated vaccine and vector strains of Vibrio cholerae. John M; Crean T I; Calderwood S B; Ryan E T. (Division of Infectious Diseases, Massachusetts General Hospital, Boston, Massachusetts 02114, USA.) INFECTION AND IMMUNITY, (2000 Mar) 68 (3) 1171-5. Journal code: 0246127. ISSN: 0019-9567. Pub. country: United States. Language: English.

AB The optimal promoter for in vivo expression of heterologous antigens by live, attenuated vaccine vector strains of Vibrio cholerae is unclear; in vitro analyses of promoter activity may not accurately predict expression of antigens in vivo. We therefore introduced plasmids expressing the B subunit of cholera toxin (**CtxB**) under the control of a number of promoters into V. cholerae vaccine strain Peru2. We evaluated the tac promoter, which is constitutively expressed in V. cholerae, as well as the in vivo-induced V. cholerae heat shock htpG promoter and the in vivo-induced V. cholerae iron-regulated irgA promoter. The functionality of all promoters was confirmed in vitro. In vitro antigenic expression was highest in vaccine strains expressing **CtxB** under the control of the tac promoter (2 to 5 microgram/ml/unit of optical density at 600 nm [OD(600)]) and, under low-iron conditions, in strains containing the irgA promoter (5 microgram/ml/OD(600)). We orally inoculated mice with the various vaccine strains and used anti-**CtxB** immune responses as a marker for in vivo expression of **CtxB**. The vaccine strain expressing **CtxB** under the control of the tac promoter elicited the most prominent specific anti-**CtxB** responses in vivo (serum immunoglobulin G [IgG], P </= 0.05; serum IgA, P </= 0.05; stool IgA, P </= 0.05; bile IgA, P </= 0.05), despite the finding that the tac and irgA promoters expressed equivalent amounts of **CtxB** in vitro. Vibriocidal antibody titers were equivalent in all groups of animals. Our results indicate that in vitro assessment of antigen expression by vaccine and vector strains of V. cholerae may correlate poorly with immune responses in vivo and that of the promoters examined, the tac promoter may be best suited for expression from plasmids of at least certain heterologous antigens in such strains.

L20 ANSWER 30 OF 128 MEDLINE DUPLICATE 21

2000072688 Document Number: 20072688. PubMed ID: 10603391. Development of

a DeltaglnA balanced lethal plasmid system for expression of heterologous antigens by attenuated vaccine vector strains of Vibrio cholerae. Ryan E T; Crean T I; Kochi S K; John M; Luciano A A; Killeen K P; Klose K E; Calderwood S B. (Division of Infectious Diseases, Massachusetts General Hospital, Boston, Massachusetts 02114, USA.) INFECTION AND IMMUNITY, (2000 Jan) 68 (1) 221-6. Journal code: 0246127. ISSN: 0019-9567. Pub. country: United States. Language: English.

AB We have previously shown that more prominent immune responses are induced to antigens expressed from multicopy plasmids in live attenuated vaccine vector strains of Vibrio cholerae than to antigens expressed from single-copy genes on the V. cholerae chromosome. Here, we report the construction of a DeltaglnA derivative of V. cholerae vaccine strain Peru2. This mutant strain, Peru2DeltaglnA, is unable to grow on medium that does not contain glutamine; this growth deficiency is complemented by pKEK71-NotI, a plasmid containing a complete copy of the Salmonella typhimurium glnA gene, or by pTIC5, a derivative of pKEK71-NotI containing a 1. 8-kbp fragment that directs expression of **CtxB** with a 12-amino-acid epitope of the serine-rich Entamoeba histolytica protein fused to the amino terminus. Strain Peru2DeltaglnA(pTIC5) produced 10-fold more SREHP-12-**CtxB** in supernatants than did ETR3, a Peru2-derivative strain containing the same fragment inserted on the chromosome. To assess immune responses to antigens expressed by this balanced lethal system in vivo, we inoculated germfree mice on days 0, 14, 28, and 42 with Peru2DeltaglnA, Peru2DeltaglnA(pKEK71-NotI), Peru2(pTIC5), Peru2DeltaglnA(pTIC5), or ETR3. All V. cholerae strains were recoverable from stool for 8 to 12 days after primary inoculation, including Peru2DeltaglnA; strains containing plasmids continued to harbor pKEK71-NotI or pTIC5 for 8 to 10 days after primary inoculation. Animals were sacrificed on day 56, and serum, stool and biliary samples were analyzed for immune responses. Vibriocidal antibody responses, reflective of in vivo colonization, were equivalent in all groups of animals. However, specific anti-**CtxB** immune responses in serum (P </= 0.05) and bile (P </= 0. 001) were significantly higher in animals that received Peru2DeltaglnA(pTIC5) than in those that received ETR3, confirming the advantage of higher-level antigen expression in vivo. The development of this balanced lethal system thus permits construction and maintenance of vaccine and vector strains of V. cholerae that express high levels of immunogenic antigens from plasmid vectors without the need for antibiotic selection pressure.

L20 ANSWER 31 OF 128 SCISEARCH COPYRIGHT 2002 ISI (R)DUPLICATE 22

2000:233305 The Genuine Article (R) Number: 295EY. Cloning of the **CtxB** gene of Vibrio cholerae and its expression in E. coli. He Z Y (Reprint); Li M F; Zhang W J; Wu X F. CHINESE ACAD SCI, SHANGHAI INST BIOCHEM, SHANGHAI 200031, PEOPLES R CHINA; SHANGHAI JIAO TONG UNIV, DEPT BIOSCI & BIOTECHNOL, SHANGHAI 200240, PEOPLES R CHINA. ACTA BIOCHIMICA ET BIOPHYSICA SINICA (MAR 2000) Vol. 32, No. 2, pp. 149-152. Publisher: SHANGHAI INST BIOCHEMISTRY, ACADEMIA SINICA. 320 YUE-YANG ROAD, SHANGHAI 20031, PEOPLES R CHINA. ISSN: 0582-9879. Pub. country: PEOPLES R CHINA. Language: Chinese.

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB The **CtxB** gene encoding cholerae toxin subunit B was amplified from Vibrio cholerae genomic DNA by PCR. The result of sequencing indicated that **CtxB** gene encodes: 123 amino acid residues. The sequence of **CtxB** gene was almost the same as that of reported except for the codon of Thr 62. The expression plasmid pGEX-**CTXB** was constructed by inserting **CtxB** gene into plasmid pGEX-4T-2, containing fist gene, immediately downstream of the T7 promoter. The expressed plasmid was introduced into E. coli BL21(DE3) cells and expression strain **CTXB**/BL21 was selected. SDS-PAGE analysis revealed that the GST-**CTXB** fusion protein was highly expression and accumulated up to 36% of bacterial soluble proteins after the induction by IPTG. A fusion protein of 40 kD was expressed as inclusion

body. The fusion protein was refolded and purified. The purified fusion protein was cut by thrombin to obtain the purified **CTXB** protein.

L20 ANSWER 32 OF 128 CAPLUS COPYRIGHT 2002 ACS
2000:486637 Document No. 134:249344 Regulation mechanism of proportional expression of cholera toxin subunits A and B genes. Cao, Cheng; Li, Ping; Li, Jiezhi; Shi, Chenghua; Ma, Qingjun (Institute of Biotechnology, Academy of Military Medical Sciences, Beijing, 100850, Peop. Rep. China). Junshi Yixue Kexueyuan Yuankan, 24(2), 88-90 (Chinese) 2000. CODEN: JYKYEL. ISSN: 1000-5501. Publisher: Junshi Yixue Kexueyuan Yuankan Bianjibu.

AB The translation efficacy from the internal translation regulation element in cholera toxin subunit A gene was studied and the regulation by translation from the initiation of ctxA gene was analyzed. One of the 3 internal translation initiation regions (TIR) was chem. synthesized and cloned into the report plasmid, and the expression of the report gene was studied. When the TIR sequence was inserted between the TIR of upstream gene and the report gene, the expression level of the report gene was doubled; When the ATG of the upstream gene was changed to ATC, no translation occurred from the internal TIR. The translation initiation efficacy from TIR-1 was same as that from the upstream gene and was controlled by the TIR from the upstream region.

L20 ANSWER 33 OF 128 CAPLUS COPYRIGHT 2002 ACS
2000:486636 Document No. 134:247715 Construction of .DELTA.asd mutant of Shigella flexneri 2a strain T32. Wang, Hengliang; Feng, Erling; Lin, Yun; Liao, Xiang; Su, Guofu (Beijing Institute of Biotechnology, Beijing, 100071, Peop. Rep. China). Junshi Yixue Kexueyuan Yuankan, 24(2), 81-87 (Chinese) 2000. CODEN: JYKYEL. ISSN: 1000-5501. Publisher: Junshi Yixue Kexueyuan Yuankan Bianjibu.

AB The aspartic semialdehyde dehydrogenase (asd) mutant of S. flexneri 2a strain T32 was constructed. Asd gene and its 3',5' chromosomal DNA sequences (-500 bp, resp.) were obtained from S.flexneri 2a strain T32 by whole-cell PCR, cloned into pUC18, and sequenced. In vitro, asd gene was replaced by **ctxB** gene, and then the **ctxB** with asd's flanks was inserted into the suicide vector pXL275. By bacterial mating and in vivo homologous recombination, asd gene of S.flexneri 2a strain T32 was completely replaced by **ctxB** gene. An asd mutant of T32, FWL01, was constructed. This mutant could express **ctxB** stably. The basis for constructing a multivalent vaccine with the vector/host balanced lethal systems in the future was provided.

L20 ANSWER 34 OF 128 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.DUPLICATE 23
2000114046 EMBASE Expression and characterization of Helicobacter pylori adhesin protein linked to cholera toxin A2/B subunits in Escherichia coli. Kim B.O.; Sung Seup Shin; Young Hyo Yoo; Pyo S.. S. Pyo, School of Pharmacy, Sung Kyun Kwan University, Suwon 440-746, Korea, Republic of. snpyo@yurim.skku.ac.kr. Journal of Microbiology and Biotechnology 10/1 (56-62) 2000.
Refs: 28.
ISSN: 1017-7825. CODEN: JOMBES. Pub. Country: Korea, Republic of.
Language: English. Summary Language: English.

AB The hpa gene genetically linked to the ctxa2b gene was cloned into the pTED expression vector, and the constructed pTEDhpa/ctxa2b was transformed into Escherichia coli. The fusion protein, the adhesin fused to the cholera toxin subunit A2B (CTXA2B) subunit, was expressed to high levels as inclusion bodies in E. coli. The expressed protein was partially purified by washing the inclusion bodies with working solution containing 8 M Urea and 0.1 M DTT. Refolding of denatured fusion protein was carried out in the presence of glutathione redox buffer. The refolded fusion protein was purified by size exclusion chromatography. The expressed fusion protein was verified by SDS- PAGE, western blotting with antibodies to both antigenic components of adhesin and cholera toxin subunit B (

CTXB), and its N-terminal amino acid sequence was analyzed. The orderly assembled fusion protein was confirmed by modified G(MI)-ganglioside ELISA with Abs to adhesin. The results indicate that the purified fusion protein is an Adhesin/CTXA2B protein containing the H. pylori adhesin and G(MI)-ganglioside binding activity of **CTXB** and the expressed fusion protein in E. coli could be easily purified by the refolding process. Its molecular weight was 168 kDa as estimated by size exclusion chromatography. The Adhesin/CTXA2B protein may be used as a candidate antigen for oral immunization against H. pylori.

L20 ANSWER 35 OF 128 CAPLUS COPYRIGHT 2002 ACS
2002:420039 Efforts towards the development of oral cholera vaccines in India against the backdrop of global endeavours. Ghosh, Amit; Thungapathra, M.; Sharma, C.; Gupta, N.; Ghosh, R. K.; Mukhopadhyay, A.; Kole, H.; Nair, G. B. (Institute of Microbial Technology, Chandigarh, 160 036, India). Diarrhoeal Diseases: Research Perspectives, [Lectures delivered at the Symposium on "New Perspectives of Research in Cholera and Diarrhoeal Diseases"], New Delhi, India, Mar. 18, 1998, Meeting Date 1998, 1-16. Editor(s): Rao, N. Appaji; Ganguly, N. K. Indian National Science Academy: New Delhi, India. ISBN: 81-7319-343-6 (English) 2000. CODEN: 69CQUD.

AB According to the World Health Organization more than 70 million people died of infectious diseases in 1996. One of the infectious diseases which continues to cause global concern is cholera. Though it can be controlled by improved sanitation, this goal is not attainable in most countries of the world. The development of an effective vaccine therefore, still remains the best soln. As parenteral vaccines developed during the last 100 yr were found to be ineffective, in recent years two different approaches have been pursued for developing oral vaccines. While the first approach is based on the observation that a cholera patient develops both anti-toxic and anti-bacterial immunity, the rationale for the second approach is that convalescents develop lasting immunity against a fresh attack. Using the first approach, a combination vaccine comprising killed whole cells and the B subunit of cholera toxin, was developed. The outcome of the second approach was the development of ctxA-B+ strains of Vibrio cholerae (strains unable to synthesize the catalytic A subunit of the cholera toxin), which could mimic infection derived immunity in the host, when administered orally. However, all such strains were found to be reactogenic. A crit. anal. of the available data indicated to us that the starting strain dets. the reactogenecity of the final construct. Hence a strain with requisite properties -completely non-reactogenic, devoid of all toxin genes and a good colonizer, was obtained after screening hundreds of isolates. The **ctxB** gene with its own up and downstream regulatory sequences, was then introduced into the chromosome of this strain at a specific locus. The recombinant strain designated VA1.3, was found to be completely non-reactogenic and 100% protective in animal studies. It was also found to be completely safe in toxicity studies conducted at the Post-Graduate Institute of Medical Education and Research, Chandigarh, under the guidance of Prof. N.K. Ganguly. The vaccine is now undergoing phase I trial. Approaches somewhat similar to the aforementioned ones have also been pursued by other groups in India. Thus while, Dr. B.S. Srivastava and his group (Central Drug Research Institute, Lucknow) have developed a potential subunit vaccine which looks promising, Dr. J. Das and coworkers (Indian Institute of Chem. Biol., Calcutta) have developed a plasmid based recombinant oral vaccine which has produced good results in animal studies.

L20 ANSWER 36 OF 128 CAPLUS COPYRIGHT 2002 ACS
1999:736498 Document No. 131:335799 Immunomodulatory activity of B subunits of cholera toxin, verotoxin, and heat-labile enterotoxin. Hirst, Timothy Raymond; Williams, Neil Andrew (University of Bristol, UK). PCT Int. Appl. WO 9958145 A2 19991118, 63 pp. DESIGNATED STATES: W: AE, AL, AM,

AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1999-GB1461 19990510. PRIORITY: GB 1998-9958 19980508; GB 1998-11954 19980603; GB 1998-12316 19980608.

AB The authors disclose the use of: (i) heat-labile enterotoxin B subunit (EtxB), cholera toxin B subunit (**CtxB**) or verotoxin B subunit (VtxB) in vaccine prepns. to alter the immune response to pathogens. In one example, the secretory IgA response to herpes virus glycoproteins is enhanced by the adjuvant activity of EtxB. In addn., the authors disclose the use of agents other than EtxB or **CtxB**, which have ganglioside GM1-binding activity, or an agent other than VtxB which has globotriosylceramide (Gb3)-binding activity for affecting intracellular signaling events.

L20 ANSWER 37 OF 128 CAPLUS COPYRIGHT 2002 ACS

1999:388084 Document No. 131:43574 An over-expressing homologous antigen vaccine and a method of making the same. Boyle, Stephen M.; Cravero, Silvio; Corbeil, Lynette; Schurig, Gerhardt G.; Srirnaganathan, Nammalwar; Vemulapalli, Ramesh (Virginia Tech Intellectual Properties, Inc., USA). PCT Int. Appl. WO 9929340 A1 19990617, 37 pp. DESIGNATED STATES: W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1997-US23032 19971205.

AB This invention relates to an over-expressing homologous antigen vaccine, a method of producing the same, and use of the vaccine for prophylaxis or treatment of vertebrates at risk of or suffering form disease caused by a pathogenic micro-organism. The vaccine is an attenuated or avirulent pathogenic micro-organism that over-expresses at least one homologous antigen encoded by at least one gene derived form the pathogenic micro-organism, and may also express a heterologous antigen. Brucella abortus over-expressing Cu/Zn SOD and GroES and GroEL genes was prepd. as vaccine for treatment of Brucellosis in animal, esp. bovine. Also, Vibrio cholera over-expressing **ctxB** gene and Mycobacterium tuberculosis over-expressing GroEL gene were prepd. as vaccines.

L20 ANSWER 38 OF 128 CAPLUS COPYRIGHT 2002 ACS

1999:193820 Document No. 130:220304 Avirulent strains of Vibrio cholerae 01 and non-01 serogroups for use in live vaccines and their construction. Kaper, James B.; Levine, Myron M. (The University of Maryland System, USA). U.S. US 5882653 A 19990316, 63 pp., Cont.-in-part of U.S. Ser. No. 133,438, abandoned. (English). CODEN: USXXAM. APPLICATION: US 1996-624601 19960729. PRIORITY: US 1983-472276 19830304; US 1984-581406 19840217; US 1986-867633 19860527; US 1989-363383 19890605; US 1990-533315 19900605; US 1992-821872 19920116; US 1992-931943 19920812; US 1993-133438 19931008; US 1993-133439 19931008; WO 1994-US11424 19941007.

AB Avirulent Vibrio cholerae strains of O1 (CVD111) and non-O1 (CVD112 and CVD112RM) serogroups suitable for vaccine use are described. These strains are non-toxigenic as a result of having the DNA for the cholera toxin core and the RS1 sequences of the cholera toxin locus deleted, and further having a mercury resistance marker and a sequence encoding an antigenic fragment of the toxin B subunit re-inserted in the chromosome. Further, these strains may also have the zona occludens toxin and accessory cholera enterotoxin genes deleted and retain the ability to colonize the small intestine. Methods of making the avirulent V. cholerae

O1 and non-O1 strains of the invention, and cholera vaccines using these strains. Although remaining antigenic, these strains do not appear to be reactogenic.

L20 ANSWER 39 OF 128 CAPLUS COPYRIGHT 2002 ACS
1999:457943 Document No. 131:78415 Nontoxinogenic cholera organisms and vaccines. Muthukumarappa, Thungapathra; Ghosh, Amit; Sharma, Charu; Gupta, Naveen; Ghosh, Ranajit K.; Mukhopadhyay, A.; Kole, Hemanta; Nair, G. B. (Council of Scientific and Industrial Research, India; National Institute of Cholera and Enteric Diseases; Department of Biotechnology, Ministry of Science and Technology, Government of India). Eur. Pat. Appl. EP 928831 A1 19990714, 18 pp. DESIGNATED STATES: R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO. (English). CODEN: EPXXDW. APPLICATION: EP 1997-309957 19971210.
AB A process for the isolation of nontoxinogenic V. cholerae strain and a process for prepg. a cholera vaccine from said V. cholerae strain is provided. V. cholerae is isolated from the stool of a patient suffering from cholera by spreading the stool on a selector medium specific for V. cholerae.1. The non-toxinogenic V. cholerae strain is sepd. from the population of the V. cholerae strains isolated in the first step. The strain is deposited at Microbial Type Culture Collection (MTCC) at the Institute of Microbial Technol. (IMT), Chandigarh, India, a constituent lab. of the applicants and has the Accession no. MTCC B0010 and is also deposited at American Type Culture Collection, Rockville, Maryland, USA with the Accession no. ATCC 202010. Immunogenic cholera toxin (ctx) B subunit gene is incorporated into the chromosomal gene hlyA (encoding hemolysin A) by genetic recombination of the strain having the Accession no. MTCC B0010 (ATCC 202010) to produce the vaccine.

L20 ANSWER 40 OF 128 CAPLUS COPYRIGHT 2002 ACS
1999:768680 Document No. 132:62844 Randomized, double-blind, placebo-controlled, multicentered trial of the efficacy of a single dose of live oral cholera vaccine CVD 103-HgR in preventing cholera following challenge with Vibrio cholerae O1 El Tor inaba three months after vaccination. Tacket, Carol O.; Cohen, Mitchell B.; Wasserman, Steven S.; Losonsky, Genevieve; Livio, Sofie; Kotloff, Karen; Edelman, Robert; Kaper, James B.; Cryz, Stanley J.; Giannella, Ralph A.; Schiff, Gilbert; Levine, Myron M. (Center for Vaccine Development, University of Maryland School of Medicine, Baltimore, MD, 21201, USA). Infection and Immunity, 67(12), 6341-6345 (English) 1999. CODEN: INFIBR. ISSN: 0019-9567. Publisher: American Society for Microbiology.
AB CVD 103-HgR is a live oral cholera vaccine strain constructed by deleting 94% of the gene for the enzymically active A subunit of cholera toxin from classical Inaba Vibrio cholerae O1 569B; the strain also contains a mercury resistance gene as an identifying marker. This vaccine was well tolerated and immunogenic in double-blind, controlled studies and was protective in open-label studies of volunteers challenged with V. cholerae O1. A randomized, double-blind, placebo-controlled, multicenter study of vaccine efficacy was designed to test longer-term protection of CVD 103-HgR against moderate and severe El Tor cholera in U.S. volunteers. A total of 85 volunteers (50 at the University of Maryland and 35 at Children's Hospital Medical Center/University of Cincinnati) were recruited for vaccination and challenge with wild-type V. cholerae El Tor Inaba. Volunteers were randomized in a double-blind manner to receive, with buffer, a single oral dose of either CVD 103-HgR (2.times.108 to 8.times.108 CFU) or placebo (killed E. coli K-12). About 3 mo after immunization, 51 of these volunteers were orally challenged with 105 CFU of virulent V. cholerae O1 El Tor Inaba strain N16961, prepd. from a standardized frozen inoculum. Ninety-one percent of the vaccinees had a .gtoreq.4-fold rise in serum vibriocidal antibodies after vaccination. After challenge, 9 (39%) of the 23 placebo recipients and 1 (4%) of the 28 vaccinees had moderate or severe diarrhea (.gtoreq.3-L diarrheal stool) (protective efficacy, 91%). A total of 21 (91%) of 23 placebo recipients

and 5 (18%) of 28 vaccinees had any diarrhea (protective efficacy, 80%). Peak stool V. cholerae excretion among placebo recipients was 1.1.times.107 CFU/g and among vaccinees was 4.9.times.102 CFU/g. This vaccine could therefore be a safe and effective tool to prevent cholera in travelers.

L20 ANSWER 41 OF 128 CAPLUS COPYRIGHT 2002 ACS
1999:291175 Document No. 131:112302 Transient transcriptional activation of the Vibrio cholerae El Tor virulence regulator ToxT in response to culture conditions. Medrano, Ana I.; DiRita, Victor J.; Castillo, Gabriela; Sanchez, Joaquin (Facultad de Medicina, UAEM, Morelos, 62210, Mex.). Infection and Immunity, 67(5), 2178-2183 (English) 1999. CODEN: INFIBR. ISSN: 0019-9567. Publisher: American Society for Microbiology.
AB Vibrio cholerae El Tor require special in vitro culture conditions, consisting of an initial static growth period followed by shift to shaking (AKI conditions), for expression of cholera toxin (CT) and toxin coregulated pili (TCP). ToxT, a regulator whose initial transcription depends on the ToxR regulator, pos. modulates expression of CT and TCP. To help understand control of CT and TCP in El Tor vibrios, we monitored ctxAB and ToxR-dependent toxT transcription by time course primer extension assays. AKI conditions stimulated CT synthesis with an absence of ctxAB transcription during static growth followed by induction upon shaking. ToxR-dependent toxT transcription was induced at the end of the static growth period but was transient, stopping shortly after shaking was initiated but, interestingly, also if the static phase was prolonged. Immunoblot assays showed that ToxR protein levels were not coincidentally transient, implying a protein on/off switch mechanism for ToxR. Despite the transient activation by ToxR, transcription of ctxAB was maintained during shaking. This finding suggested continued toxT expression, possibly through relay transcription from another promoter. The 12.6-kb distant upstream tcpA promoter responsible for expression of the TCP operon has been proposed to provide an alternate toxT message by readthrough transcription. Activation of the tcpA promoter is supported by increased expression of TcpA protein during the shaking phase of the culture. Readthrough transcription of toxT from tcpA would be compatible with reverse transcription-PCR evidence for a toxT mRNA at times when ToxR-dependent transcription was no longer detectable by primer extension.

L20 ANSWER 42 OF 128 CAPLUS COPYRIGHT 2002 ACS
2000:45414 Document No. 133:38973 Translation coupling effect on gene expression in operon of cholera toxin. Cao, Cheng; Li, Jiezhi; Wang, Xiao; Li, Ping; Shi, Chenghua; Ma, Qingjun (Institute of Biotechnology, Academy of Military Medical Sciences, Beijing, 100850, Peop. Rep. China). Junshi Yixue Kexueyuan Yuankan, 23(4), 241-244 (Chinese) 1999. CODEN: JYKYEL. ISSN: 1000-5501. Publisher: Junshi Yixue Kexueyuan Yuankan Bianjibu.
AB The translation coupling between cholera toxin A subunit gene and B subunit gene was studied; the expression level of subunit B gene being 5 times more than that of the subunit A gene. A reporter system for the translation coupling was constructed by using lacZ gene as the reporter, and frame-shift mutation was introduced near the C terminal of ctxA gene so the ribosome would read through its normal stop codon during translation. The expression level of lacZ gene decreased by 5 times after the frame-shift mutation. Thus, the translation of cholera toxin B subunit gene was coupled with A subunit gene by translation coupling, and was responsible for the differential expression level of the two genes.

L20 ANSWER 43 OF 128 MEDLINE DUPLICATE 24
1999351710 Document Number: 99351710. PubMed ID: 10424424. Construction of a recombinant live oral vaccine from a non-toxigenic strain of Vibrio cholerae O1 serotype inaba biotype El Tor and assessment of its reactogenicity and immunogenicity in the rabbit model. Thungapathra M; Sharma C; Gupta N; Ghosh R K; Mukhopadhyay A; Koley H; Nair G B; Ghosh A.

(Institute of Microbial Technology, Sector 39A, Chandigarh, India.) IMMUNOLOGY LETTERS, (1999 Jun 1) 68 (2-3) 219-27. Journal code: 7910006. ISSN: 0165-2478. Pub. country: Netherlands. Language: English.

AB The disease cholera is an important cause of mortality in many developing countries. Though it can be controlled through improved sanitation, this goal is not easily attainable in many countries. Development of an efficacious vaccine offers the best immediate solution. A new oral candidate vaccine has been constructed from a non-toxigenic strain of Vibrio cholerae E1 Tor, Inaba, which is not only devoid of the cholera toxin (CT) virulence cassette but also is completely non-reactogenic in rabbit ileal loop assay. The strain, however, had toxR and tcpA genes. Through a series of manipulations, the **ctxB** gene of V. cholerae, responsible for the production of the 'B' subunit of the cholera toxin (CTB) was introduced into the cryptic hemolysin locus of the strain. The resulting strain, named vaccine attempt 1.3 (VA1.3), was found to be able to produce copious amounts of CTB. In the RITARD model this strain was found to be non-reactogenic and provided full protection against the challenge doses of both V. cholerae O1, classical and E1 Tor. In the immunized rabbit it invoked significant levels of anti-bacterial and anti-toxin immunity.

L20 ANSWER 44 OF 128 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC. DUPLICATE 25

1999:356089 Document No.: PREV199900356089. Genome of bacteriophage CTXPHI without the presence of ctxAB exists in ctxAB- strains of Vibrio cholerae. Kan, Biao (1); Qi, Guoming (1); Liu, Yanqing (1). (1) The Priority Laboratory of Medical Molecular Bacteriology of the Ministry of Public Health, Institut, Chinese Academy of Preventive Medicine, Beijing, 102206 China. Zhonghua Weishengwuxue He Mianyixue Zazhi, (May, 1999) Vol. 19, No. 3, pp. 175-179. ISSN: 0254-5101. Language: Chinese. Summary Language: Chinese; English.

AB Objective To research on the lysogenic bacteriophage CTXPHI genome, for isogenous sequences with RS in the chromosomes were found in some strains of ctxAB- E1 Tor biotype (EVC) isolates in this lab. Methods The DNA probes of CTXPHI genes, including ctxA, **ctxB**, zot, ace, cep and RS, were prepared and were used to detect the corresponding genes in the ctxAB- strains with Southern blot analysis. The major gene of CTXPHI receptor Tcp (toxin-coregulated pilus), tcpA, was detected by polymerase chain reaction analysis. Results CTXPHI genome in the chromosomes were found in 4 isolates of ctxAB- EVC strains from water, and 1 isolate from a patient only possessed RS sequence. tcpA of all these strains was positive. Conclusions The results suggest that ctxAB may not has a concurrent occurrence with other CTXPHI genes. A novel CTXPHI genome without ctxAB gene presents in the chromosome of V. cholerae.

L20 ANSWER 45 OF 128 MEDLINE DUPLICATE 26

1998389309 Document Number: 98389309. PubMed ID: 9723916. Evidence that a globular conformation is not compatible with FhaC-mediated secretion of the Bordetella pertussis filamentous haemagglutinin. Guedin S; Willery E; Locht C; Jacob-Dubuisson F. (INSERM U447, IBL, Institut Pasteur de Lille, France.) MOLECULAR MICROBIOLOGY, (1998 Aug) 29 (3) 763-74. Journal code: 8712028. ISSN: 0950-382X. Pub. country: ENGLAND: United Kingdom. Language: English.

AB The 220 kDa Bordetella pertussis filamentous haemagglutinin (FHA) is the major extracellular protein of this organism. It is exported using a signal peptide-dependent pathway, and its secretion depends on one specific outer membrane accessory protein, FhaC. In this work, we have investigated the influence of conformation on the FhaC-mediated secretion of FHA using an 80kDa N-terminal FHA derivative, Fha44. In contrast to many signal peptide-dependent secretory proteins, no soluble periplasmic intermediate of Fha44 could be isolated. In addition, cell-associated Fha44 synthesized in the absence of FhaC did not remain competent for extracellular secretion upon delayed expression of FhaC, indicating that

the translocation steps across the cytoplasmic and the outer membrane might be coupled. A chimeric protein, in which the globular B subunit of the cholera toxin, **CtxB**, was fused at the C-terminus of Fha44, was not secreted in B. pertussis or in Escherichia coli expressing FhaC. The hybrid protein was only secreted when both disulphide bond-forming cysteines of **CtxB** were replaced by serines or when it was produced in DsbA- E. coli. The Fha44 portion of the secretion-incompetent hybrid protein was partly exposed on the cell surface. These results argue that the Fha44-**CtxB** hybrid protein transited through the periplasmic space, where disulphide bond formation is specifically catalysed, and that secretion across the outer membrane was initiated. The folded **CtxB** portion prevented extracellular release of the hybrid, in contrast to the more flexible **CtxB** domain devoid of cysteines. We propose a secretion model whereby Fha44 transits through the periplasmic space on its way to the cell surface and initiates its translocation through the outer membrane before being released from the cytoplasmic membrane. Coupling of Fha44 translocation across both membranes would delay the acquisition of its folded structure until the protein emerges from the outer membrane. Such a model would be consistent with the extensive intracellular proteolysis of FHA derivatives in B. pertussis.

L20 ANSWER 46 OF 128 CAPLUS COPYRIGHT 2002 ACS
1998:573955 Document No. 129:311600 Diverse CTX.PHI.s and evolution of new pathogenic Vibrio cholerae. Kimsey, Harvey H.; Nair, G. Balakrish; Ghosh, Amit; Waldor, Matthew K. (Division of Geographic Medicine and Infectious Diseases, Tufts-New England Medical Center, Boston, MA, 02111, USA). Lancet, 352(9126), 457-458 (English) 1998. CODEN: LANCAO. ISSN: 0140-6736. Publisher: Lancet Ltd..

AB The immunity region of the CTX element from a reemerged Vibrio cholerae O139 strain, O139 Calcutta, was sequenced and compared with an O130 Bengal strain. O139 Calcutta harbors an El-Tor-type CTX prophage (CTX.PHI.ImmEl Tor) as well as a unique O139 Calcutta prophage (CTX.PHI.Imm Calcutta) with a novel immunity region. The evolution of the O139 Calcutta via phage-mediated horizontal gene transfer in the development of new pathogens is discussed.

L20 ANSWER 47 OF 128 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
1999:144553 Document No.: PREV199900144553. Receptor mediated apoptosis of CD89+T cells by the B subunits of cholera-like enterotoxins. Pitman, Richard S.; Hirst, Timothy R.; Nashar, Toufic O.; Williams, Neil A.. Dep. Pathol. Microbiol., Sch. Med. Sci., Univ. Bristol, Bristol BS8 1TD UK. Biochemical Society Transactions, (Nov., 1998) Vol. 26, No. 4, pp. S338. Meeting Info.: 666th Meeting of the Biochemical Society Sheffield, England, UK July 29-31, 1998 ISSN: 0300-5127. Language: English.

L20 ANSWER 48 OF 128 CAPLUS COPYRIGHT 2002 ACS
1999:373884 Document No. 131:154427 The regulation of cholera toxin gene expression. Duan, Guangcai; Rappuoli, Rino; Gao, Shouyi; Liu, Yanqing; Qi, Guoming; Fontana, MariaRita (Department of Epidemiology, Henan Medical University, Zhengzhou, 450052, Peop. Rep. China). Henan Yike Daxue Xuebao, 33(5), 62-65 (Chinese) 1998. CODEN: HEYDE2. ISSN: 1000-1069. Publisher: Henan Yike Daxue Xuebao Bianjibu.

AB Cholera toxin (CT) consists of one A subunit (CT-A) and five B subunits (CT-B). The genes encoding A subunit and B subunit are named ctxA and **ctxB**. Conventional studies indicated that the transcription of ctxA and **ctxB** is one open reading frame (ORF) in the control of the promoter Pctx. In this study, we constructed several high CT expressing vectors in order to develop the new cholera vaccines. The vaccine candidate strain IEM101 (Vibrio cholerae O1, CT neg.) was transformed with these plasmids by electro-transformation, and the expression levels of CT-A and CT-B were detected with western blot. CT-B could be expressed together with CT-A under the control of different

promoter systems; meanwhile, CT-B could also be expressed well even when CT-A was not expressed. Apparently, there is a promoter-like system at the upper stream of **ctxB**, and CT-B can be expressed independently. The effects of different promoters on CT expression in Vibrio cholerae are also studied. The promoters Ptac, PlacUV5, PT7 could work well in IEM101.

L20 ANSWER 49 OF 128 MEDLINE DUPLICATE 27
1998062149 Document Number: 98062149. PubMed ID: 9400977. Salmonella typhimurium aroA recombinants and immune-stimulating complexes as vaccine candidates for feline immunodeficiency virus. Tijhaar E J; Huisman W; Huisman R C; Siebelink K H; Karlas J A; de Ronde A; van Herwijnen R; Mooi F R; Osterhaus A D. (National Institute of Public Health and the Environment, Bilthoven, The Netherlands.) JOURNAL OF GENERAL VIROLOGY, (1997 Dec) 78 (Pt 12) 3265-75. Journal code: 0077340. ISSN: 0022-1317. Pub. country: ENGLAND: United Kingdom. Language: English.

AB Two experimental feline immunodeficiency virus (FIV) vaccines were tested, either alone or in combination, in four groups of cats (A-D). One vaccine (SL3261-FIV) was composed of live attenuated Salmonella typhimurium aroA (SL3261) strains expressing the capsid (Gag) and part of the envelope (Env) proteins of FIV. The other was composed of FIV Gag and Env proteins incorporated into immune-stimulating complexes (iscom-FIV). Cats of group A were immunized four times with SL3261-FIV. Cats of group B were immunized twice with SL3261-FIV and then twice with iscom-FIV. Cats of group C were immunized twice with SL3261 expressing the B subunit of cholera toxin (SL3261-**CtxB**) and then twice with iscom-FIV. Cats of group D, which served as negative controls, were immunized twice with SL3261-**CtxB** and then twice with iscom into which the Gag and Env proteins of simian immunodeficiency virus (SIV) had been incorporated (iscom-SIV). Two weeks after the last immunization, all cats were challenged with FIV. At this time, cats immunized with iscom-FIV (groups B and C) showed strong plasma antibody responses to Gag and Env, whilst these responses were weak or undetectable in the cats immunized four times with SL3261-FIV (group A). Seven weeks after FIV challenge, Env-specific antibody responses had increased considerably in cats of all groups except group A. The mean virus loads in the cats of this group proved to be lower than those of the other groups at all time points, indicating partial protection.

L20 ANSWER 50 OF 128 MEDLINE DUPLICATE 28
97230341 Document Number: 97230341. PubMed ID: 9119505. Promoter activities in Vibrio cholerae ctx phi prophage. Fando R; Perez J L; Rodriguez B L; Campos J; Robert A; Garcia L; Silva A; Benitez J A. (Centro Nacional de Investigaciones Cientificas, Havana, Cuba.) INFECTION AND IMMUNITY, (1997 Apr) 65 (4) 1561-5. Journal code: 0246127. ISSN: 0019-9567. Pub. country: United States. Language: English.

AB Comparison of cholera toxin (CT) production directed by different gene constructs and S1 nuclease mapping revealed the presence of a **ctxB**-specific promoter within the ctxA coding sequence. Initiation of transcription in this region occurred in wild-type El Tor and classical biotype choleragenic vibrios. We propose that transcription from the **ctxB**-specific promoter and a stronger ribosomal binding site on the **ctxB** mRNA synergistically contribute to achieve the correct (5B:1A) subunit stoichiometry. Plasmid pB, a CT promoterless vector expressing only CTB, was used to detect promoter activity by restoration of A-subunit synthesis. Promoter activity expressed in vitro and in vivo was detected upstream of the zonula occludens toxin gene, suggesting that this factor could be produced in vivo to contribute to fluid accumulation. No promoter activity was detected in vitro and in vivo upstream from the accessory cholera enterotoxin gene.

L20 ANSWER 51 OF 128 MEDLINE DUPLICATE 29
97321753 Document Number: 97321753. PubMed ID: 9178455. Induction of

feline immunodeficiency virus specific antibodies in cats with an attenuated Salmonella strain expressing the Gag protein. Tijhaar E J; Siebelink K H; Karlas J A; Burger M C; Mooi F R; Osterhaus A D. (School of Biological and Medical Sciences, University of St Andrews, Scotland.) VACCINE, (1997 Apr-May) 15 (6-7) 587-96. Journal code: 8406899. ISSN: 0264-410X. Pub. country: ENGLAND: United Kingdom. Language: English.

AB Salmonella typhimurium aroA strains (SL3261), expressing high levels of the Gag protein of feline immunodeficiency virus (FIV) fused with maltose binding protein (SL3261-MFG), were constructed using an invertible promoter system that allows the stable expression of heterologous antigens at levels toxic for bacteria. A SL3261 strain expressing the B subunit of cholera toxin by a similar system (SL3261-**CtxB**) served as a control in FIV-immunization experiments. Cats immunized once orally or intraperitoneally with SL3261-MFG or SL3261-**CtxB** all developed serum antibodies to SL3261 lipopolysaccharide and against maltose binding protein or the B subunit of cholera toxin, respectively. Two intraperitoneal immunizations with SL3261-MFG also resulted in the development of Gag specific serum antibodies. Two oral immunizations with SL3261-MFG primed for a Gag specific response, which was demonstrated upon FIV challenge. All challenged cats became infected and no significant differences in viral loads were found between SL3261-MFG and SL3261-**CtxB** immunized cats.

L20 ANSWER 52 OF 128 MEDLINE DUPLICATE 30

1998018503 Document Number: 98018503. PubMed ID: 9378497. Modulation of B-cell activation by the B subunit of Escherichia coli enterotoxin: receptor interaction up-regulates MHC class II, B7, CD40, CD25 and ICAM-1. Nashar T O; Hirst T R; Williams N A. (School of Medical Sciences, University of Bristol, UK.) IMMUNOLOGY, (1997 Aug) 91 (4) 572-8. Journal code: 0374672. ISSN: 0019-2805. Pub. country: ENGLAND: United Kingdom. Language: English.

AB The B subunits of cholera toxin (**CtxB**) and Escherichia coli heat-labile enterotoxin (EtxB) are non-toxic lectins that bind and cross-link a ubiquitous cell glycolipid receptor, ganglioside GM1, and are recognized as potent mucosal and systemic immunogens. Here we examine the role of EtxB receptor occupancy in modulating the activation of B cells, in vitro, in primary lymphocyte cultures containing B and T cells. When 48-hr spleen cell cultures containing EtxB were compared with those in the presence of a non-receptor binding mutant, EtxB(G33D), a marked shift in the ratio of CD4+ T cells: B cells was noted. Evidence suggested that this was the result of either enhanced survival or proliferation of B cells associated with receptor occupancy by EtxB. Investigation revealed that EtxB induced only a minimal increase in proliferation above that of EtxB(G33D), in mixed cell cultures, and failed to induce any cell division of purified B cells or T cells. In contrast, receptor-binding by EtxB markedly up-regulated the expression of major histocompatability complex (MHC) class II, B7, intracellular adhesion molecule-1 (ICAM-1), CD40 and CD25 on the B-cell surface. These results indicate that the polyclonal effects of EtxB on B cells are not associated with wide-scale proliferation, but more likely with maintenance of B-cell survival by activation of molecules essential for B-cell differentiation. The findings also highlight the essential role of GM1-interaction with EtxB in the regulation of lymphocyte responses.

L20 ANSWER 53 OF 128 CAPLUS COPYRIGHT 2002 ACS

1997:470984 Document No. 127:157367 Gene fusion and expression of lipoprotein with cholera toxin B subunit and hepatitis B virus Pres2 epitope. Lin, Xu; Shi, Cheng-Hua; Cao, Cheng; Li, Ping; Bao, You-Di; Ma, Qing-Jun (Inst. Biotechnol., Acad. Millitary Med. Sci., Beijing, 100850, Peop. Rep. China). Shengwu Huaxue Zazhi, 13(3), 276-281 (Chinese) 1997. CODEN: SHZAE4. ISSN: 1000-8543. Publisher: Zhongguo Shengwu Huaxue Yu Fenzi Shengwu Xuehui.

AB Lipoprotein (lpp) of the outer membrane of Escherichia coli is a major

protein of the cell wall. The N-terminal amino acids of lipoprotein and its artificial analogs were promising adjuvant in construction of peptide vaccine for their activity activating B-lymphocyte, macrophage and CTL. Nucleotides encoding signal peptides and N-terminal nine amino acids (LPP9) of lipoprotein were genetically fused to the 5' end of the **ctxB**-Pres2 gene by means of PCR. The fusion protein was well expressed with relatively low yield (about 0, 1-0.2 mg/l), and anchored in cell membrane. The fusion protein obtained in this way was more convenient and economical than chem. conjugation. The chimera was modified correctly and retained the biol. activity of both CTB and Pres2 as confirmed by 3H-palmitic acid labeling test and GM I Enzyme Linked Immunosorbent Assay resp. The signal peptide of lipoprotein was important to the correct modification of the chimera, and **ctxB** promoter was more efficient than that of lpp on the expression of chimers.

L20 ANSWER 54 OF 128 MEDLINE DUPLICATE 31
97323775 Document Number: 97323775. PubMed ID: 9312275. [Toxigenic Vibrio cholerae: identification of the **ctxB** gene]. Vibrio cholerae toxigenico: identificacion del gen **ctxB**. Vergara M; Maestre J; Suarez O; Monte R. (Catedra de Bacteriologia, Facultad de Ciencias Exactas Quimicas y Naturales, Universidad Nacional de Misiones, Argentina.) ENFERMEDADES INFECCIOSAS Y MICROBIOLOGIA CLINICA, (1997 Apr) 15 (4) 181-5. Journal code: 9104081. ISSN: 0213-005X. Pub. country: Spain. Language: Spanish.

AB BACKGROUND: A specific probe was designed to identify part of the genetic sequence of the **ctxB** gene which encodes for the B subunit of the cholera toxin by polymerase chain reaction (PCR) which amplifies a 318 bp segment of the **ctxB** gene. Marked with P32, we used this probe for colony hybridization which is a technique for identifying the production capacity of subunit B of strains of Vibrio cholerae O1 from different outbreaks in South America (Peru 1992 and Ecuador 1993-1995) and from, collection strains. This probe was tested for the identification of the **ctxB** gene in Vibrio cholerae O139. METHOD: Thirty-eight phylogenetically related strains were studied: 24 V. cholerae O1, 4 V. cholerae non O1, 5 Aeromonas, 4 Plesiomonas and 1 Escherichia coli. RESULTS: The probe demonstrated to be useful for the identification of the **ctxB** gene (which codifies for the subunit B of the cholera toxin) in 24 strains of Vibrio cholerae O1 and in the Vibrio cholerae O139 strain. The **ctxB** gene was not detected in the remaining strains pertaining to the Vibrio cholerae non O1 species (non O139), Plesiomonas, Aeromonas spp. and E. coli. The specificity of this product was not demonstrated since no signal of unspecific hybridization appeared with phylogenetically related strains such as Escherichia coli K88 (LT+) and Aeromonas hydrophila ATCC (LT+), producers of the thermolabile LT toxin. It is important to indicate that the **ctxB** gene in V. cholerae O139 has been identified, for the first time, with our probe and thus it may be said that all the strains which have genetic codification for CT up to now may be identified. CONCLUSIONS: We conclude that the system herein described provides advantages over the immunologic and biologic methods for evaluating a large number of samples in a short time and with excellent specificity and sensitivity which are important in the diagnosis and the epidemiologic surveillance of the disease.

L20 ANSWER 55 OF 128 CAPLUS COPYRIGHT 2002 ACS
1998:26759 Document No. 128:150086 Construction and characterization of versatile cloning vectors for efficient delivery of native foreign proteins to the periplasm of Escherichia coli. Jobling, Michael G.; Palmer, Leslie M.; Erbe, Jarrod L.; Holmes, Randall K. (Dep. Microbiol., Univ. Colorado Health Sci. Cent., Denver, CO, 80262, USA). Plasmid, 38(3), 158-173 (English) 1997. CODEN: PLSMDX. ISSN: 0147-619X. Publisher: Academic Press.

AB Induction of the wild type cholera toxin operon (ctxAB) from multicopy clones in Escherichia coli inhibited growth and resulted in low yields of

cholera toxin (CT). We found that prodn. of wild type CT or its B subunit (CT-B) as a periplasmic protein was toxic for E. coli, but by replacing the native signal sequences of both CT-A and CT-B with the signal sequence from the B subunit of E. coli heat-labile enterotoxin LTIIb we succeeded for the first time in producing CT holotoxin in high yield in E. coli. Based on these findings, we designed and constructed versatile cloning vectors that use the LTIIb-B signal sequence to direct recombinant native proteins with high efficiency to the periplasm of E. coli. We confirmed the usefulness of these vectors by producing two other secreted recombinant proteins. First, using phoA from E. coli, we demonstrated that alk. phosphatase activity was 17-fold greater when the LTIIb-B signal sequence was used than when the native leader for alk. phosphatase was used. Second, using the pspA gene that encodes pneumococcal surface protein A from Streptococcus pneumoniae, we produced a 299-residue amino-terminal fragment of PspA in E. coli in large amts. as a sol. periplasmic protein and showed that it was immunoreactive in Western blot with antibodies against native PspA. The vectors described here will be useful for further studies on structure-function relationships and vaccine development with CT and PspA, and they should be valuable as general tools for delivery of other secretion-competent recombinant proteins to the periplasm in E. coli.

L20 ANSWER 56 OF 128 CAPLUS COPYRIGHT 2002 ACS
1997:431131 Document No. 127:120453 Immunogenicity of CT-B::DTx-B, CT-B::PT-S1*,S2, and CT-B::TT-B chimeric proteins: an approach to develop a safer DPT vaccine. Lu, Ying; Peterson, J.W.; Chopra, A.K. (Department of Microbiology and Immunology, The University of Texas Medical Branch, Galveston, TX, 77555-1019, USA). Vaccine Research, 6(1), 1-13 (English) 1997. CODEN: VAREES. ISSN: 1056-7909. Publisher: Liebert.

AB The authors report here the construction of several chimeric genes encoding the binding domains of diphtheria (DTx-B), pertussis (PT-S2), and tetanus (TT-B) toxins, as well as a modified PT-S1 enzymic subunit, which were placed downstream and in-frame with the immunomodulatory cholera toxin B-subunit (CT-B) gene. Each chimeric gene construct was hyperexpressed in Escherichia coli, and the fusion proteins, i.e., CT-B::DTx-B, CT-B::PT-S2, and CT-B::TT-B, reacted with antibodies to each component of the chimeras in an ELISA and Western blot anal. The hyperproduced proteins induced significant antibody titers in mice against both components of the chimeric proteins. A mutagenized PT-S1 subunit gene construct was made by first using site-directed mutagenesis to modify codons encoding amino acid residues Arg9 and Glu129, which are responsible for the ADP-ribosyltransferase activity of the PT-S1 subunit. The codons for these amino acids were replaced with those encoding Lys and Gly, resp., and the genetically inactivated PT-S1* gene was ligated downstream of the CT-B gene and expressed in E. coli. After hyperexpression, antibodies generated against the CT-B::PT-S1* construct neutralized the Chinese hamster ovary (CHO) cell clustering activity, which is a typical PT biol. response. Further, the antibodies blocked the elongation of CHO cells, which is a characteristic response of these cells to CT. These chimeric antigens may be beneficial in the development of alternative recombinant vaccines with minimal toxic side effects compared with those seen with the whole cell DPT vaccine.

L20 ANSWER 57 OF 128 MEDLINE DUPLICATE 32
96325012 Document Number: 96325012. PubMed ID: 8702586. Assembly of the B subunit pentamer of Escherichia coli heat-labile enterotoxin. Kinetics and molecular basis of rate-limiting steps in vitro. Ruddock L W; Coen J J; Cheesman C; Freedman R B; Hirst T R. (Research School of Biosciences, University of Kent, Canterbury, Kent CT2 7NJ, United Kingdom.) JOURNAL OF BIOLOGICAL CHEMISTRY, (1996 Aug 9) 271 (32) 19118-23. Journal code: 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.

AB The B subunits of Escherichia coli heat-labile enterotoxin (EtxB) and cholera toxin (**CtxB**) assemble in vivo into exceptionally stable

homopentameric complexes, which maintain their quaternary structure in a range of conditions that would normally be expected to cause protein denaturation. Recently, we showed that the simultaneous protonation of two of the COOH-terminal carboxylates in pentameric EtxB was required to cause its disassembly at pH values below 2.0 (Ruddock, L., Ruston, S. P., Kelly, S. M., Price, N. C., Freedman, R. B., and Hirst, T. R.(1995) J. Biol. Chem. 270, 29953-29958). Here, we investigate the influence of environmental parameters on the kinetics of reassembly of acid-generated EtxB monomers in vitro. Such monomers were found to undergo a further acid-mediated conformational change, with an activation energy of 76 +/- 2 J.mol-1.K-1, consistent with isomerization of the cis-proline residue at position 93, and which prevented subsequent EtxB reassembly. By using rapid neutralization of acid-generated monomers, a high proportion of the B-subunits adopted an assembly-competent conformation, which resulted in up to 75% of the protein reassembling into a stable pentameric complex, indistinguishable from native EtxB pentamers. The rate-limiting step in reassembly, over a concentration range of 50-200 microg/ml, was shown to be due to an intramolecular event, which exhibited a pH dependence with a pKa of 7.0. Modification of EtxB with amine-specific probes revealed that the protonation state of the NH2-terminal alanine residue was responsible for the pH dependence of reassembly. The implications of these findings for the biogenesis of Escherichia coli enterotoxin and related enterotoxins in vivo, are considered.

L20 ANSWER 58 OF 128 MEDLINE DUPLICATE 33

96390007 Document Number: 96390007. PubMed ID: 8797101. Expression of the cholera toxin B subunit in the Golgi apparatus of Swiss 3T3 cells inhibits DNA synthesis induced by basic fibroblast growth factor. Hashimoto Y; Oshima A; Narimatsu H; Suzuki A. (Department of Membrane Biochemistry, Tokyo Metropolitan Institute of Medical Science.) JOURNAL OF BIOCHEMISTRY, (1996 May) 119 (5) 985-90. Journal code: 0376600. ISSN: 0021-924X. Pub. country: Japan. Language: English.

AB We attempted to express the cholera toxin B subunit (**CTXB**) in the Golgi apparatus of cultured mammalian cells by means of gene transfection. Complementary DNA of **CTXB** was ligated with the Golgi-retention signal sequence of human beta 1,4 galactosyltransferase cDNA, and the chimeric gene yielded was inserted into a mammalian expression vector. The resultant construct was transfected into COS-1 cells for transient expression and into Swiss 3T3 cells for stable expression. The expression of a fusion protein encoded by the chimeric gene was demonstrated according to the following criteria: first, detection of a protein exhibiting the expected molecular mass on Western blot analysis using an anti-**CTXB** antibody; second, detection of the protein located in the Golgi area by indirect immunofluoresence microscopy; and third, detection of GM1 binding activity in cell lysates. Stable transformants satisfying the above criteria were subjected to an assay for mitogen-induced DNA synthesis. These transformants exhibited significantly lower DNA synthesis than mock transfection cells on stimulation with basic fibroblast growth factor (bFGF), whereas the two types of cells exhibited similar responses to 10% fetal calf serum and other mitogens, such as epidermal growth factor, 12-O-tetradecanoylphorbol-13-acetate, calcium ionophore A23187, and platelet-derived growth factor. Analysis of the binding of radio-iodinated bFGF to the cells revealed that the transformants did not exhibit a significant decrease in the binding affinity or the number of high affinity sites. These results suggest that the fusion protein specifically inhibits the bFGF signaling not at the binding step but rather at a later step(s) triggered by the binding.

L20 ANSWER 59 OF 128 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.

1997:29106 Document No.: PREV199799328309. ELISA for the detection of LT enterotoxin of E. coli strains. Osek, Jacek. ul. Kosciuszki 12/24, 24-100 Pulawy Poland. Medycyna Weterynaryjna, (1996) Vol. 52, No. 11, pp. 705-707. ISSN: 0025-8628. Language: Polish. Summary Language: English.

AB The aim of the study was to develop a sensitive and specific ELISA for the detection of LT enterotoxin in E. coli strains. The test was based on the antitoxic antibodies obtained from rabbits immunized with B subunit of cholera toxin (CT). The toxin was structurally closely related with LTB of E. coli. CTB was expressed in a V. cholerae strain harboring a plasmid with a **ctxB** gene. The toxin was isolated and purified from a growth medium by means of precipitation. A goat antirabbit IgG labeled with HRP antibodies was used as a conjugate. The developed test turned out to be highly specific and sensitive: it was possible to detect from 1.5 to 4.0 ng of LTB. One hundred strains of E. coli isolated from piglets were tested by the LT ELISA. It was found that 67 strains (85.9%) isolated from piglets with diarrhea were LT+ whereas only 2 strains (9.1%) from healthy piglets proved to be enterotoxigenic.

L20 ANSWER 60 OF 128 CAPLUS COPYRIGHT 2002 ACS
1996:597666 Document No. 125:239643 Genetic manipulation of Vibrio cholerae for vaccine development: construction of live attenuated El Tor candidate vaccine strains. Benitez, Jorge A.; Silva, Anisia J.; Rodriguez, Boris L.; Fando, Rafael; Campos, Javier; Robert, Alma; Garcia, Hilda; Garcia, Luis; Perez, Jose Luis; et al. (Grupo de Genetica del Centro Nacional de Investigaciones Cientificas, Havana, Cuba). Arch. Med. Res., 27(3), 275-283 (English) 1996. CODEN: AEDEER. ISSN: 0188-4409.

AB The recent spread of El Tor cholera to America augments the need for an effective, safe and economical vaccine. In the present paper we describe the construction of live attenuated V. cholerae strains by specifically deleting the genes encoding cholera toxin and other putative toxins from the bacterial chromosome. To maximize the likelihood of exposing protective antigens relevant to currently circulating vibrios we selected for genetic manipulation recent epidemic V. cholerae isolates from Peru. The mutant strains did not produce cholera toxin in vitro and in vivo. Deletion of the virulence cassette was accompanied by marked attenuation in the infant mouse cholera model. A selected El Tor Ogawa candidate vaccine strain was refractory to acquisition of foreign genes by conjugation with toxigenic vibrios.

L20 ANSWER 61 OF 128 CAPLUS COPYRIGHT 2002 ACS
1996:274128 Document No. 124:338741 Trophic effect of cholera toxin B subunit in cultured cerebellar granule neurons: modulation of intracellular calcium by GM1 ganglioside. Wu, G.; Lu, Z.-H.; Nakamura, K.; Spray, D. C.; Ledeen, R. W. (Dep. Neurosciences, New Jersey Medical School of UMDNJ, Newark, NJ, 07103, USA). J. Neurosci. Res., 44(3), 243-254 (English) 1996. CODEN: JNREDK. ISSN: 0360-4012.

AB Survival of cerebellar granule cells (CGC) in culture was significantly improved in the presence of cholera toxin B subunit (**CtxB**), a ligand which binds to GM1 with specificity and high affinity. This trophic effect was linked to elevation of intracellular calcium ([Ca2+]i), and was additive to that of high K+. Survival was optimized when **CtxB** was present for several days during the early culture period. 45Ca2+ and cell survival studies indicated the mechanism to involve enhanced influx of Ca2+ through L-type voltage-sensitive channels, since the trophic effect was blocked by antagonists specific for that channel type. Inhibitors of N-methyl-D-aspartate receptor/channels were without effect. During the early stage of culture **CtxB**, together with 25 mM K+, caused [Ca2+]i to rise to 0.2-0.7 .mu.M in a higher proportion of cells than 25 mM K+ alone. A significant change in the nature of GM1 modulation of Ca2+ flux occurred after 7 days in culture, at which time **CtxB** ceased to elevate and instead reduced [Ca2+]i below the level attained with 25 mM K+. GM1 thus appears to serve as intrinsic inhibitor of one or more L-type Ca2+ channels during the first 7 days in vitro, and then as intrinsic activator of (possibly other) L-type channels after that period. This is the first demonstration of a modulatory role for GM1 ganglioside affecting Ca2+ homeostasis in cultured neurons of the CNS.

L20 ANSWER 62 OF 128 CAPLUS COPYRIGHT 2002 ACS
1996:252992 Document No. 124:281050 Genetic footprint of the ToxR-binding site in the promoter for cholera toxin. Pfau, James D.; Taylor, Ronald K. (Dep. Microbil., Dartmouth Medical School, Hannover, NH, 03755, USA). Mol. Microbiol., 20(1), 213-22 (English) 1996. CODEN: MOMIEE. ISSN: 0950-382X.

AB The transmembrane DNA-binding protein, ToxR, of Vibrio cholerae is a global transcriptional regulator of virulence gene expression. ToxR has been shown to interact with promoter regions upstream of both the ctxAB operon encoding cholera toxin, and the regulatory gene toxT. Deletion anal. has shown that a repeated sequence, TTTTGAT, is required for ToxR binding and activation of the ctxAB promoter. However, this sequence is not found upstream of the toxT promoter. Genetic selections using P22 challenge phages were used to define sites within the promoter **ctxB** which are crit. for ToxR-DNA interactions. Single-base-pair changes and deletion mutations that impair ToxR binding cluster within two regions: -57 to -69 within two of three tandem TTTTGAT sequences; and the -35 region of the promoter. ToxR does not bind to a synthetic target that has three tandem repeats which lack a flanking upstream and downstream sequence. These results suggest that the ToxR-binding site lies immediately upstream of the -35 position of the ctx promoter, and that the affinity of ToxR binding to this site is influenced by the repeat sequences.

L20 ANSWER 63 OF 128 MEDLINE
1999021860 Document Number: 99021860. PubMed ID: 9805044. [The application of the hybridization in colonies technic for the identification of toxigenic Vibrio cholerae 01]. Aplicacion de la tecnica de hibridacion en colonias para la identificacion de Vibrio cholerae 01 toxigenico. Bravo Farinas L; Monte Boada R J; Ramirez Alvarez M; Maestre Mesa J L; Suarez Moreno O; Morales Grillo J. (Instituto de Medicina Tropical Pedro Kouri.) REVISTA CUBANA DE MEDICINA TROPICAL, (1996) 48 (3) 169-70. Journal code: 0074364. ISSN: 0375-0760. Pub. country: Cuba. Language: Spanish.

AB By means of the polymerase chain reaction (PCR) it was obtained a probe for the gen that codifies the subunit B of cholerae toxin (**CTxB**), which carried a Vibrio cholerae 01 reference strain. The checking of the amplified product was performed by using the hybridization techniques in colonies. This product hybridized with the gen that codifies for the subunit B of cholerae toxin isolated from Peru and Ecuador, representing the present epidemics in Latin America, but it did not so with the phylogenetically related strains.

L20 ANSWER 64 OF 128 MEDLINE DUPLICATE 34
97001662 Document Number: 97001662. PubMed ID: 8844654. Vibrio cholerae hemagglutinin/protease (HA/protease) causes morphological changes in cultured epithelial cells and perturbs their paracellular barrier function. Wu Z; Milton D; Nybom P; Sjo A; Magnusson K E. (Department of Medical Microbiology, Linkoping University, Sweden.) MICROBIAL PATHOGENESIS, (1996 Aug) 21 (2) 111-23. Journal code: 8606191. ISSN: 0882-4010. Pub. country: ENGLAND: United Kingdom. Language: English.

AB In this report, we describe the cytotoxic activity of the cholera hemagglutinin/protease (HA/protease). A concentrated protein sample from the 37 degrees C overnight culture supernatant of CVD110, a delta ctxA, delta zot, delta Ace and hlyA::(**ctxB** mer) mutant of El Tor biotype Ogawa serotype strain E7946 caused morphological changes in cultured MDCK-I epithelial cells and altered their arrangement of filamentous actin (F-actin) and Zonula occludens-associated protein ZO-1. The drastic morphological changes can be inhibited by Zincov, a specific bacterial metalloprotease inhibitor. The cytotoxic fractions of the sample after FPLC gelfiltration fractionation showed two visible protein bands with molecular weights of approximately 34- and 32 kDa. Microsequencing of these two proteins revealed that they were the cholera HA/protease.

L20 ANSWER 65 OF 128 MEDLINE DUPLICATE 35
97080554 Document Number: 97080554. PubMed ID: 8921899. Absence of periplasmic DsbA oxidoreductase facilitates export of cysteine-containing passenger proteins to the Escherichia coli cell surface via the Iga beta autotransporter pathway. Jose J; Kramer J; Klauser T; Pohlner J; Meyer T F. (Max-Planck-Institut fur Biologie, Abteilung Infektionsbiologie, Tubingen, Germany.) GENE, (1996 Oct 31) 178 (1-2) 107-10. Journal code: 7706761. ISSN: 0378-1119. Pub. country: Netherlands. Language: English.

AB The Iga beta autotransporter function of IgA1 protease from Neisseria gonorrhoeae was assessed in Escherichia coli using the Vibrio cholerae toxin B subunit (**CtxB**) as a heterologous passenger. N-terminal fusions with Iga beta of native **CtxB** or mutant **CtxB** protein containing no cysteines were constructed and analysed in isogenic E. coli mutants carrying defects in either or both the ompT (outer membrane protease T) and dsbA (periplasmic disulfide oxidoreductase) determinants. While export of the cystein-less **CtxB** passenger was independent of the dsbA genotype, the native **CtxB** passenger was properly translocated across the outer membrane only in the dsbA mutant background. This effect was consistent in the presence and in the absence of the OmpT protease which rather determined the release of surface-bound **CtxB** into the medium. Therefore, in agreement with previous observations Iga beta-dependent protein secretion requires an unfolded conformation of the passenger domain and can be blocked by disulfide loop formation in the presence of DsbA. Since DsbA acts in the periplasm, this provides evidence for a periplasmic intermediate in the Iga beta-mediated export pathway. E. coli (dsbA ompT) is highly suitable as a strain for the surface display of recombinant proteins via Iga beta, whether or not they contain cysteine residues.

L20 ANSWER 66 OF 128 CAPLUS COPYRIGHT 2002 ACS
1995:804342 Document No. 123:196581 Vibrio cholerae 01 (CVD111) and non-01 (CVD112 and CVD112rm) serogroup vaccine strains and methods of making same. Kaper, James B.; Levine, Myron M. (University of Maryland, USA). PCT Int. Appl. WO 9510300 A1 19950420, 109 pp. DESIGNATED STATES: W: AU, CA, CN, JP, KR, RU, US; RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE. (English). CODEN: PIXXD2. APPLICATION: WO 1994-US11424 19941007. PRIORITY: US 1993-133438 19931008; US 1993-133439 19931008.

AB Avirulent Vibrio cholerae strains of 01(CVD111) and non-01 (CVD112 and CVD112RM) serogroups having the DNA of the cholera toxin core and the RS1 sequences of the cholera toxin locus deleted, and further having a DNA encoding a resistance to mercury, and a DNA encoding the cholera toxin B subunit, or a part thereof sufficient to confer immunogenicity, re-inserted in the chromosome. Methods of making the avirulent V. cholerae 01 and non-01 strains of the invention, and cholera vaccines using these strains.

L20 ANSWER 67 OF 128 CAPLUS COPYRIGHT 2002 ACS
1996:30105 Document No. 124:97719 Isolating restriction fragment deletions in Vibrio cholerae, and vaccine products with toxin gene deletions. Kaper, James B.; Baudry Maurelli, Bernadette; Fasano, Alessio (University of Maryland, USA). U.S. US 5470729 A 19951128, 55 pp. Cont.-in-part of U.S. Ser. No. 821,872, abandoned. (English). CODEN: USXXAM. APPLICATION: US 1992-931943 19920812. PRIORITY: US 1983-472276 19830304; US 1984-581406 19840217; US 1986-867633 19860527; US 1989-363383 19890605; US 1990-533315 19900605; US 1992-821872 19920116.

AB Methods of isolating deletion mutants of Vibrio cholerae are described. In one method, the deletion is predetd. by digestion with restriction endonucleases of known specificity. The deletions are inserted into the Vibrio cholerae chromosome by in vivo recombination between a plasmid carrying the desired deletion, with adjacent flanking sequences, and the Vibrio cholerae chromosome. In another method, an initial in vivo

recombination event of homologous sequences from the recombinant plasmid into the chromosome provides a selectable marker at this site. A second in vivo recombination event between homologous flanking sequences results in excision of proficient genes from the chromosome with the end product being a deletion mutation. Also provided are methods for the isolation and characterization of a new Vibrio cholerae strain having a deletion in the ctx gene, as defined by AccI, XbaI, ClaI, and/or other restriction endonuclease sites and further having a deletion in the gene encoding zonula occludens toxin (zot). Thus, a culture of Vibrio cholerae was constructed with a deletion in the cholera toxin A and B subunit genes (ctxA and **ctxB**), which confers avirulence and retains the capacity to colonize the intestine of a host animal. A second culture deletes the zonula occludens toxin gene (zot) in addn. to the ctx genes, in order to reduce residual diarrhea in the host animal. Another culture has a region of chromosomal DNA coding for cholera toxin and zonula occludens toxin deleted, and having inserted a mercury resistance gene and DNA coding for B subunit of Vibrio toxin. These strains are avirulent without affecting other components necessary for immunity; they confer substantially close to 100% efficacy in humans against subsequent disease with a strain of a similar serotype and avoid undesirable side effects such as diarrhea and nausea and cramping. The ctx and zot gene deletions include the gene for ACE (accessory cholera enterotoxin), whose DNA sequence was detd.

L20 ANSWER 68 OF 128 MEDLINE DUPLICATE 36

96102052 Document Number: 96102052. PubMed ID: 8530395. Kinetics of acid-mediated disassembly of the B subunit pentamer of Escherichia coli heat-labile enterotoxin. Molecular basis of pH stability. Ruddock L W; Ruston S P; Kelly S M; Price N C; Freedman R B; Hirst T R. (Biological Laboratory, University of Kent, Canterbury, United Kingdom.) JOURNAL OF BIOLOGICAL CHEMISTRY, (1995 Dec 15) 270 (50) 29953-8. Journal code: 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.

AB The B-subunit pentamer of Escherichia coli heat-labile enterotoxin (EtxB) is highly stable, maintaining its quaternary structure in a range of conditions that would normally be expected to cause protein denaturation. In this paper the structural stability of EtxB has been studied as a function of pH by electrophoretic, immunochemical, and spectroscopic techniques. Disassembly of the cyclic pentameric structure of human EtxB occurs only below pH 2. As determined by changes in intrinsic fluorescence this process follows first-order kinetics, with the rate constant for disassembly being proportional to the square of the H+ ion concentration, and with an activation energy of 155 kJ mol-1. A C-terminal deletion mutant, hEtxB214, similarly shows first-order kinetics for disassembly but with a higher pH threshold, resulting in disassembly being seen at pH 3.4 and below. These findings are consistent with the rate-limiting step for disassembly of human EtxB being the simultaneous disruption of two interfaces by protonation of two C-terminal carboxylates. By comparison, disassembly of the B-subunit of cholera toxin (**CtxB**), a protein which shows 80% sequence identity with EtxB, exhibits a much lower stability to acid conditions; with disassembly of **CtxB** occurring below pH 3.9, with an activation energy of 81 kJ mol-1. Reasons for the observed differences in acid stability are discussed, and the implications of these findings to the development of oral vaccines using EtxB and **CtxB** are considered.

L20 ANSWER 69 OF 128 MEDLINE DUPLICATE 37

95393966 Document Number: 95393966. PubMed ID: 7664730. Membrane insertion of the bacterial signal transduction protein ToxR and requirements of transcription activation studied by modular replacement of different protein substructures. Kolmar H; Hennecke F; Gotze K; Janzer B; Vogt B; Mayer F; Fritz H J. (Institut fur Molekulare Genetik, George-August-Universitat Gottingen, Germany.) EMBO JOURNAL, (1995 Aug 15) 14 (16) 3895-904. Journal code: 8208664. ISSN: 0261-4189. Pub.

country: ENGLAND: United Kingdom. Language: English.

AB The Vibrio cholerae protein ToxR is an integral membrane protein that acts as a transcription activator in response to environmental signals; it controls expression of toxin genes ctxA and **ctxB**, along with a variety of other genes related to pathogenicity. Here it is shown that: (i) ToxR has a modular architecture and that activation of transcription starting at the ctx promoter depends strictly on dimerization of the periplasmic ToxR domain; (ii) the transmembrane (TM) region of ToxR is sufficient as a topogenic signal but not for stable membrane anchoring of the protein; (iii) the TM region has no special function in signal transduction and (iv) a proline residue located within the TM region minimizes background transcription activation, most plausibly by reducing TM-TM interaction. Possible applications of ToxR as a technical tool for analysing protein-protein interactions between pairs of arbitrary TM domains are discussed.

L20 ANSWER 70 OF 128 MEDLINE DUPLICATE 38

95310029 Document Number: 95310029. PubMed ID: 7790086. Heterologous antigen expression in Vibrio cholerae vector strains. Butterton J R; Beattie D T; Gardel C L; Carroll P A; Hyman T; Killeen K P; Mekalanos J J; Calderwood S B. (Infectious Disease Unit, Massachusetts General Hospital, Boston 02114, USA.) INFECTION AND IMMUNITY, (1995 Jul) 63 (7) 2689-96. Journal code: 0246127. ISSN: 0019-9567. Pub. country: United States. Language: English.

AB Live attenuated vector strains of Vibrio cholerae were derived from Peru-2, a Peruvian El Tor Inaba strain deleted for the cholera toxin genetic element and attRS1 sequences, which was developed as a live, oral vaccine strain. A promoterless gene encoding the Shiga-like toxin I B subunit (slt-IB) was inserted in the V. cholerae virulence gene irgA by in vivo marker exchange, such that slt-IB was under transcriptional control of the iron-regulated irgA promoter. slt-IB was also placed under transcriptional control of the V. cholerae heat shock promoter, htpGp, and introduced into either the irgA or lacZ locus, or both loci, on the chromosome of Peru-2, generating JRB10, JRB11, or JRB12, respectively. A new technique was used to perform allelic exchange with lacZ. This method uses plasmid p6891MCS, a pBR327 derivative containing cloned V. cholerae lacZ, to insert markers of interest into the V. cholerae chromosome. Recombinants can be detected by simple color screening and antibiotic selection. In vitro measurements of Slt-IB produced by the vector strains suggested that expression of Slt-IB from the irgA and htpG promoters was synergistic and that two copies of the gene for Slt-IB increased expression over a single copy. The V. cholerae vectors colonized the gastrointestinal mucosa of rabbits after oral immunization, as demonstrated by very high serum antibody responses to V. cholerae antigens. Comparison of the serologic responses to the B subunit of cholera toxin (**CtxB**) following orogastric inoculation either with the wild-type C6709 or with Peru-10, a strain containing **ctxB** regulated by htpGp, suggested that both the cholera toxin and heat shock promoters were active in vivo, provoking comparable immunologic responses. Orogastric inoculation of rabbits with vector strains evoked serum immunoglobulin G (IgG) responses to Slt-IB in two of the four strains tested; all four strains produced biliary IgA responses. No correlation was observed between the type of promoter expressing slt-IB and the level of serum IgG or biliary IgA response, but the vector strain containing two copies of the gene for slt-IB evoked greater serum IgG responses than strains containing a single copy, consistent with the increased expression of Slt-IB from this strain observed in vitro. A comparison of the serum and biliary antibody responses to Slt-IB expressed from htpGp versus **CtxB** expressed from the same promoter suggested that **CtxB** is a more effective orally delivered immunogen.

L20 ANSWER 71 OF 128 MEDLINE DUPLICATE 39

95197259 Document Number: 95197259. PubMed ID: 7890393. Oral immunization

with the dodecapeptide repeat of the serine-rich Entamoeba histolytica protein (SREHP) fused to the cholera toxin B subunit induces a mucosal and systemic anti-SREHP antibody response. Zhang T; Li E; Stanley S L Jr. (Department of Medicine, Washington University School of Medicine, St. Louis, Missouri 63110.) INFECTION AND IMMUNITY, (1995 Apr) 63 (4) 1349-55. Journal code: 0246127. ISSN: 0019-9567. Pub. country: United States. Language: English.

AB The intestinal protozoan parasite Entamoeba histolytica causes amebic dysentery, a major cause of morbidity worldwide. The induction of a mucosal antibody response capable of blocking amebic adhesion to intestinal cells could represent an approach to preventing E. histolytica infection and disease. Here we describe the expression of a chimeric protein containing an immunogenic dodecapeptide derived from the serine-rich E. histolytica protein (SREHP), fused to the cholera toxin B subunit (**CtxB**). The **CtxB**-SREHP-12 chimeric protein was purified from Escherichia coli lysates and retained the critical GM1 ganglioside-binding activity of the **CtxB** moiety. Mice fed the **CtxB**-SREHP-12 fusion protein along with a subclinical dose of cholera toxin developed mucosal immunoglobulin A and immunoglobulin G and systemic antibody responses that recognized recombinant and native SREHP. Our study confirms the feasibility of inducing mucosal immune responses to immunogenic peptides by their genetic fusion to the **CtxB** subunit and identifies the **CtxB**-SREHP-12 chimeric protein as a candidate oral vaccine to prevent E. histolytica infection.

L20 ANSWER 72 OF 128 MEDLINE DUPLICATE 40

96126032 Document Number: 96126032. PubMed ID: 8589171. Primary septicemia caused by Vibrio cholerae non-O1 acquired on Cape Cod, Massachusetts. Kontoyiannis D P; Calia K E; Basgoz N; Calderwood S B. (Infectious Disease Unit, Massachusetts General Hospital, Boston 02114, USA.) CLINICAL INFECTIOUS DISEASES, (1995 Nov) 21 (5) 1330-3. Journal code: 9203213. ISSN: 1058-4838. Pub. country: United States. Language: English.

AB We describe a patient with non-O1, non-O139 Vibrio cholerae septicemia associated with hemorrhagic bullous skin lesions of the lower extremities. The patient had underlying liver disease, and he probably acquired the organism through ingestion of raw clams. Although his condition rapidly improved during appropriate therapy, the patient's cellulitis and skin lesions persisted and he developed a fluid collection of the lower extremity that required drainage. Molecular methods were used to examine the non-O1 V. cholerae isolate for several known virulence factors of V. cholerae O1. The isolate failed to express cholera toxin and toxin-coregulated pilus (Tcp) and was negative in Southern hybridizations for **ctxB**, tcpA, toxR, and toxT. The vast majority of vibrio infections in the United States are clustered in the Gulf Coast area. This patient acquired the infection on Cape Cod. To our knowledge, this is the first case of non-O1 V. cholerae septicemia reported to have occurred in Massachusetts. Given the high fatality rate of this infection, it is important for physicians to consider this diagnosis in patients who have underlying risk factors and appropriate epidemiologic exposures, even when they reside as far north as the New England states.

L20 ANSWER 73 OF 128 SCISEARCH COPYRIGHT 2002 ISI (R)

95:524300 The Genuine Article (R) Number: RL566. IMMUNOREGULATORY ROLE OF H-2 AND INTRA-H-2 ALLELES ON ANTIBODY-RESPONSES TO RECOMBINANT PREPARATIONS OF B-SUBUNITS OF ESCHERICHIA-COLI HEAT-LABILE ENTEROTOXIN (RETXB) AND CHOLERA-TOXIN (RCTXB). NASHAR T O (Reprint); HIRST T R. UNIV KENT, RES SCH BIOSCI, CANTERBURY CT2 7NJ, KENT, ENGLAND (Reprint). VACCINE (JUN 1995) Vol. 13, No. 9, pp. 803-810. ISSN: 0264-410X. Pub. country: ENGLAND. Language: ENGLISH.

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB The immunoregulatory role of H-2 and intra-H-2 alleles on antibody responses to recombinant preparations of B-subunits of Escherichia coli

heat-labile enterotoxin (rEtxB) and cholera toxin (rCtxB) is reported. Oral delivery of rEtxB to congenic mice of several different H-2 haplotypes resulted in H-2 dependent serum IgG responses (H-2(d) > H-2(b) = H-2(q) > H-2(a) > H-2(k)) and a similar spectrum of intestinal IgA responses in those strains tested Responses to rEtxB and rCtxB were found to be differentially modulated by the H-2 locus, with significant differential effects in H-2(b) and H-2(d) congenic strains (H-2(d) > H-2(b) for rEtxB; H-2(b) > H-2(d) for rCtxB), Additionally, it was found that when rEtxB was fed to mice previously primed (orally) with either rEtxB or rCtxB only those mice primed with rEtxB exhibited a booster response. A second booster immunisation with rEtxB in rCtxB-primed mice produced an H-2 dependent spectrum of responses characteristic of those elicited by rEtxB, with the antibodies predominantly directed against rEtxB and not rCtxB. These results indicate that the differential response to rEtxB and rCtxB is set at the T- and B-cell level. Also, immunoregulation of antibody responses to rEtxB by intra-H-2 I-E in mice transgenic for the entire IE(a)(k) gene was investigated. No significant difference between responses in transgene-positive and -negative mice was found, suggesting that antigen presentation does not involve I-E, but occurs in the context of I-A. The implications of these results for the design of vaccines against enterotoxigenic E. coli and cholera diarrhoea are discussed.

L20 ANSWER 74 OF 128 MEDLINE

1998448132 Document Number: 98448132. PubMed ID: 9775006. Molecular epidemiological studies of Vibrio cholerae in Taiwan: genotyping by polymerase chain reaction and DNA sequencing. Liu D P; Chen P J; Lin C S; Wu T N. (National Quarantine Service, Department of Health, Taipei, Taiwan, R.O.C.) CHUNG-HUA MIN KUO WEI SHENG WU CHI MIEN I HSUEH TSA CHIH CHINESE JOURNAL OF MICROBIOLOGY AND IMMUNOLOGY, (1995 Nov) 28 (4) 291-9. Journal code: 8008067. ISSN: 0253-2662. Pub. country: TAIWAN: Taiwan, Province of China. Language: English.

AB To type the Vibrio cholerae strains isolated from sporadic and epidemic cases in Taiwan, 28 toxigenic isolates were studied by sequencing polymerase chain reaction-amplified cholera toxin gene (ctx) fragments. Based on specific base substitutions on positions 115 and 203 of **ctxB** and comparison with previously published typing system from Centers for Disease Control and Prevention (Olsvik theta et al., J Clin Microbiol 1993; 31:22-5, Ref.1), two genotypes were identified. Cholera strains from imported seafood and sporadic cases in Taiwan had **ctxB** polymorphism of genotype 1; strains from patients in the 1962 Taiwan epidemic and Taiwan raised soft-shell turtles had **ctxB** polymorphism of genotype 3. Moreover, one toxigenic non-O1 strain was found to have other 11 different nucleotides in **ctxB** compared with those of the O1 and O139 strains. Therefore, DNA sequencing is a useful method for obtaining more complete genetic information. The approach could be improved by applying it to other more polymorphic regions of bacterial genome to obtain better epidemiological information among infected cases.

L20 ANSWER 75 OF 128 MEDLINE DUPLICATE 41

95278726 Document Number: 95278726. PubMed ID: 7758939. C-terminal glycine-histidine tagging of the outer membrane protein Iga beta of Neisseria gonorrhoeae. Strauss A; Pohlner J; Klauser T; Meyer T F. (Max-Planck-Institut fur Biologie, Abteilung Infektionsbiologie, Tubingen, Germany.) FEMS MICROBIOLOGY LETTERS, (1995 Apr 1) 127 (3) 249-54. Journal code: 7705721. ISSN: 0378-1097. Pub. country: Netherlands. Language: English.

AB A glycine-histidine tag (Gly3His6) was added to the C-terminus of a fusion protein consisting of the cholera toxin B-subunit (**CtxB**) and the IgA protease beta-domain (Iga beta). The aim was to facilitate single-step purification and to create a suitable tool for kinetic and structural studies on Iga beta-driven protein translocation across the outer membrane

of Gram-negative bacteria. We demonstrate that the glycine-histidine tag does not interfere with the assembly of Iga beta in the outer membrane and that the translocator function of the modified Iga beta is maintained. The applicability of the new construct for the dissection of the Iga beta mediated translocation process and general aspects of C-terminal histidine tagging of outer membrane proteins are discussed.

L20 ANSWER 76 OF 128 MEDLINE

95180695 Document Number: 95180695. PubMed ID: 7875567. Detection and differentiation of the gene for toxin co-regulated pili (tcpA) in Vibrio cholerae non-O1 using the polymerase chain reaction. Said B; Smith H R; Scotland S M; Rowe B. (Laboratory of Enteric Pathogens, Central Public Health Laboratory, London, UK.) FEMS MICROBIOLOGY LETTERS, (1995 Jan 15) 125 (2-3) 205-9. Journal code: 7705721. ISSN: 0378-1097. Pub. country: Netherlands. Language: English.

AB The polymerase chain reaction has been used to differentiate the gene which encodes the toxin co-regulated pili (tcpA) of the El Tor and classical biotypes of Vibrio cholerae O1. The same PCR primers were applied to strains belonging to non-O1 serogroups that produced cholera toxin. The size of fragment amplified was either identical to the tcpA of biotype El Tor (471 bp) or to the tcpA of biotype classical (617 bp). All strains belonging to the novel epidemic serogroup O139 generated a 471-bp fragment identical to El Tor tcpA. The present study suggests that there may be an association between non-O1 serogroup and tcpA type.

L20 ANSWER 77 OF 128 CAPLUS COPYRIGHT 2002 ACS

1995:847298 Document No. 124:22834 Amino acids of the cholera toxin from Vibrio cholerae O37 strain S7 which differ from those of strain O1. Yamamoto, Koichiro; Do Valle, Gloria R. F.; Xu, Ming; Miwatani, Toshio; Honda, Takeshi (Department of Bacterial Infections, Research Institute for Microbial Diseases, Osaka University, Yamadaoda, Suita, Osaka, 565, Japan). Gene, 163(1), 155-6 (English) 1995. CODEN: GENED6. ISSN: 0378-1119.

AB Unique differences in amino acid (aa) residues were found in the deduced aa sequence of cholera toxin (CT) from Vibrio cholerae non-O1 strain S7, some of which are suggested to be important sites for the unusual oligomer formation of subunit B.

L20 ANSWER 78 OF 128 MEDLINE

95096550 Document Number: 95096550. PubMed ID: 7528249. Molecular subtyping of toxigenic Vibrio cholerae O139 causing epidemic cholera in India and Bangladesh, 1992-1993. Popovic T; Fields P I; Olsvik O; Wells J G; Evins G M; Cameron D N; Farmer J J 3rd; Bopp C A; Wachsmuth K; Sack R B; +. (Foodborne and Diarrheal Diseases Branch, National Center for Infectious Diseases, Centers for Disease Control and Prevention, Atlanta, Georgia 30333.) JOURNAL OF INFECTIOUS DISEASES, (1995 Jan) 171 (1) 122-7. Journal code: 0413675. ISSN: 0022-1899. Pub. country: United States. Language: English.

AB Since October 1992, > 150,000 cases of cholera have been reported from India and Bangladesh; the great majority of Vibrio cholerae isolates belong to the newly established serogroup O139. To better understand the interaction of genetic and epidemiologic factors responsible for their sudden appearance and rapid spread, representative toxigenic V. cholerae O139 isolates were molecularly characterized and compared with a set of toxigenic V. cholerae O1 and non-O1/non-O139 strains. DNA sequences of the cholera toxin B subunit gene and multilocus enzyme electrophoresis markers of V. cholerae O139 strains were identical to those of V. cholerae O1 isolates of the seventh pandemic. Two distinct ribotypes and four pulsed-field gel electrophoretic patterns were observed for O139 strains. V. cholerae O139 strains were very similar to V. cholerae O1 strains of the seventh pandemic but clearly different from the toxigenic V. cholerae strains of serogroups other than O1 and O139.

L20 ANSWER 79 OF 128 MEDLINE DUPLICATE 42

95303036 Document Number: 95303036. PubMed ID: 7783690. Characterization of an enterotoxin produced by Vibrio cholerae O139. Nakashima K; Eguchi Y; Nakasone N. (Department of Bacteriology, Faculty of Medicine, University of the Ryukyus, Okinawa, Japan.) MICROBIOLOGY AND IMMUNOLOGY, (1995) 39 (2) 87-94. Journal code: 7703966. ISSN: 0385-5600. Pub. country: Japan. Language: English.

AB A cholera-like enterotoxin was purified from Vibrio cholerae O139 strain AI-1841 isolated from a diarrheal patient in Bangladesh. Its characteristics were compared with that of cholera toxins (CTs) of classical strain 569B and El Tor strain KT25. Al-1841 produced as much toxin as O1 strains. The toxins were indistinguishable in terms of their migration profiles in conventional polyacrylamide gel disc electrophoresis, sodium dodecyl sulfate-polyacrylamide gel electrophoresis and isoelectrofocusing as well as their affinity for hydroxyapatite. The skin permeability factor activity and the fluid accumulation induced in rabbit ileal loops of the toxin of AI-1841 were identical to those of the CTs. Three toxins equally reacted against anti-569B CT antiserum in Western blotting, and their B subunits formed a precipitin line against any anti-B subunit antiserum by double gel immunodiffusion. Anti-569B CTB antibody neutralized the three toxins in their PF activities and enterotoxicities. The amino acid sequence of 1841 toxin B subunit was identical with that of KT25 CTB, corresponding to the DNA sequence of **ctxB** from El Tor strains of the seventh pandemic. We concluded 1841 toxin was identical to CT of the seventh pandemic El Tor vibrios.

L20 ANSWER 80 OF 128 CAPLUS COPYRIGHT 2002 ACS

1995:227412 Document No. 122:48495 Membrane expression of heterologous genes with plasmids containing export signals from Salmonella typhimurium. Niesel, David W.; Moncrief, J. Scott; Phillips, Linda H. (Board of Regents, The University of Texas, USA). U.S. US 5356797 A 19941018, 26 pp. (English). CODEN: USXXAM. APPLICATION: US 1991-792525 19911115.

AB The invention relates to nucleic acid segments useful in the construction of expression vectors for expression of heterologous polypeptides directed to particular areas of the host cell. Selected constructs (e.g., the plasmid pZIP-OUT) direct prodn. of polypeptides to the outer membrane surface of the cell. Other constructs (e.g., plasmid pZIP-IN) direct expression of heterologous polypeptides to the inner membrane/periplasm of the host cell. Isolation of the Salmonella typhimurium DNA segments was accomplished by isolation of DNA fragments contg. phoA gene fusions resulting from random transposition of TnphoA (a Tn4 deriv. encoding Escherichia coli alk. phosphatase minus the signal sequence and expression signals, inserted into the left IS50L element). Plasmids contg. phoA gene fusions can then be used as exposition vectors. The SSP1 and the PvuII restriction sites in phoA are blunt ended sites at which inframe insertions of a gene of interest can be inserted. The resulting tribrid gene fusions contain the expression and export signals of the target gene fused inframe with the phoA and gene of interest. The system is demonstrated by fusions with the cholera toxin B subunit gene **ctxB** and a 60-kDa fragment of HIV gp120. Transformed host cells are potentially useful for the prodn. of vaccines or immunogens elicited in response to antigens expressed on the outer membranes of the host cells.

L20 ANSWER 81 OF 128 CAPLUS COPYRIGHT 2002 ACS

1996:359780 Document No. 125:28185 Cholera toxin A1-chain analogs which retain immunogenicity but lack ADP-ribosyltransferase activity. Burnette, Walter Neal; Kaslow, Harvey Robin (Amgen Inc., USA; University of Southern California). Indian IN 173616 A 19940611, 74 pp. (English). CODEN: INXXAP. APPLICATION: IN 1992-MA262 19920504.

AB Analogs of the catalytic subunit of cholera toxin are produced by of cultivating prokaryotic or eukaryotic host cells transformed and transfected with DNA vectors having DNA sequences encoding the analogs isolating the desired polypeptides in a known manner. The development of

subunits and subunit analogs of the cholera toxin by recombinant DNA techniques provides vaccine products that can retain their biol. activity and immunogenicity, and can confer protection against disease challenge. The genetically engineered modifications of the subunits result in products that retain immunogenicity, yet are reduced in, or are essentially free of enzymic activity assocd. with toxin reactogenicity. Thus, recombinant cholera toxin A1 chains (CTXA1) were synthesized in Escherichia coli under control of an optimized expression vector by std. methods. N-methionyl-B-chain was also cloned and expressed to prep. holo-toxins with full immunogenicity. An oligonucleotide linker substituted an initiating methionine codon for the signal peptide-encoded sequence of the preproA-chain-encoding DNA; this N-terminal methionyl residue is probably not processed away in the final products. A1-chain analogs were produced by site-specific mutagenesis, and the ADP ribosyltransferase activity tested for autocatalysis, for Gs.alpha. protein, and for H27 fibroblast and erythrocyte membranes. Mutagenesis of the amino acid residues at positions Arg7, His44, His70, Glu112, and Asp9, and truncation of the C-terminus (at Trp179 of the mature native CTXA sequence) resulted in diminished or essentially no ADP-ribosyltransferase activity.

L20 ANSWER 82 OF 128 MEDLINE DUPLICATE 43

94314415 Document Number: 94314415. PubMed ID: 8039872. Construction and characterization of recombinant Vibrio cholerae strains producing inactive cholera toxin analogs. Hase C C; Thai L S; Boesman-Finkelstein M; Mar V L; Burnette W N; Kaslow H R; Stevens L A; Moss J; Finkelstein R A. (Department of Molecular Microbiology and Immunology, School of Medicine, University of Missouri, Columbia 65212.) INFECTION AND IMMUNITY, (1994 Aug) 62 (8) 3051-7. Journal code: 0246127. ISSN: 0019-9567. Pub. country: United States. Language: English.

AB The catalytic A subunit of cholera toxin (CT-A) is capable of ADP-ribosylating the guanine nucleotide-binding protein, which regulates cell adenylyl cyclase, leading to the life-threatening diarrhea of cholera. Amino acids involved in the enzymatic activity of CT-A have previously been identified. By means of site-directed mutagenesis, an analog of the CT-A subunit gene was created with codon substitutions for both Arg-7 and Glu-112, each of which has been shown to produce subunits lacking ADP-ribosyltransferase activity. The mutated gene fragment was exchanged for the wild-type copy in the previously cloned ctxAB operon from El Tor biotype, Ogawa serotype Vibrio cholerae strain 3083, which produces CT-2. Further, the zonula occludens toxin gene, zot, was inactivated by an insertional mutation to create the new plasmid construct pCT-2*. Additionally, a DNA fragment encoding the B subunit of CT-1 (CT produced by classical biotype, Inaba serotype V. cholerae strain 569B) was exchanged for the homologous part in pCT-2*, resulting in the creation of pCT-1*. These plasmid constructs were introduced into the CT-negative V. cholerae mutant strain JBK70 (El Tor biotype, Inaba serotype); CT-A-B+ derivatives CVD101 and CVD103 of classical biotype Ogawa and Inaba serotype strains 395 and 569B, respectively; El Tor biotype Inaba and Ogawa serotype strains C6706 and C7258, respectively, recently isolated in Peru; and O139 (synonym Bengal) strain SG25-1 from the current epidemic in India. Recombinant toxins (CT-1* and CT-2*), partially purified from culture supernatants of transformed JBK70, were shown to be inactive on mouse Y1 adrenal tumor cells and in an in vitro ADP-ribosyltransferase assay. CT-1* and CT-2* reacted with polyclonal and monoclonal antibodies against both A and B subunits of CT. The toxin analogs reacted with antibodies against CT-A and CT-B on cellulose acetate strips and in a GM1 enzyme-linked immunosorbent assay; they reacted appropriately with B-subunit epitype-specific monoclonal antibodies in checkerboard immunoblots, and they formed precipitin bands with GM1-ganglioside in Ouchterlony tests. However, the reactions of the modified proteins with anti-A-subunit monoclonal antibodies were weaker than the reactions with wild-type holotoxins. V, cholerae strains carrying ctxA*, with either

ctxB-1 or **ctxB**-2, and inactivated zot genes were created by homologous recombination. The recombinant strains and the purified toxin analogs were inactive in the infant rabbit animal model.(ABSTRACT TRUNCATED AT 400 WORDS)

L20 ANSWER 83 OF 128 MEDLINE DUPLICATE 44

95088433 Document Number: 95088433. PubMed ID: 7995992. Development of a live, oral, attenuated vaccine against El Tor cholera. Taylor D N; Killeen K P; Hack D C; Kenner J R; Coster T S; Beattie D T; Ezzell J; Hyman T; Trofa A; Sjogren M H; +. (Division of Communicable Diseases and Immunology, Walter Reed Army Institute of Research, Washington, DC.) JOURNAL OF INFECTIOUS DISEASES, (1994 Dec) 170 (6) 1518-23. Journal code: 0413675. ISSN: 0022-1899. Pub. country: United States. Language: English.

AB Vibrio cholerae El Tor strains from Peru, Bangladesh, and Bahrain were attenuated by deletion of a genetic element that encodes virulence factors and RS1. The B subunit of ctx (**ctxB**) was reintroduced into the recA gene of the deletion mutants, rendering them unable to recombine with exogenous genetic elements and generating Peru-3, Bang-3, and Bah-3. Fifteen volunteers received one dose of various vaccine strains at 4 x 10(6) to 1 x 10(8) cfu. All strains colonized the gut. A > or = 4-fold rise in vibriocidal titer was observed in 14 volunteers, with titers of > or = 1600 in 13. Peru-3 was the least reactogenic, but 2 of 6 volunteers had loose stools. Peru-14, a filamentous motility-deficient mutant of Peru-3, was well tolerated and colonized 18 of 21 volunteers at doses of 2 x 10(6) to 1 x 10(9) cfu. Also, when 8 Peru-3 or Peru-5 vaccinees, 5 Peru-14 vaccinees, and 8 controls were challenged with 2 x 10(6) cfu V. cholerae El Tor Inaba (N16961), 11 vaccinees were protected compared with no controls. Peru-14 shows promise as a safe, effective, single-dose oral vaccine against El Tor cholera.

L20 ANSWER 84 OF 128 MEDLINE DUPLICATE 45

95066317 Document Number: 95066317. PubMed ID: 7975840. Construction and evaluation of an expression vector allowing the stable expression of foreign antigens in a Salmonella typhimurium vaccine strain. Tijhaar E J; Zheng-Xin Y; Karlas J A; Meyer T F; Stukart M J; Osterhaus A D; Mooi F R. (Laboratory of Immunobiology, National Institute of Public Health and Environmental Protection, Bilthoven, The Netherlands.) VACCINE, (1994 Aug) 12 (11) 1004-11. Journal code: 8406899. ISSN: 0264-410X. Pub. country: ENGLAND: United Kingdom. Language: English.

AB Salmonella strains have great potential as live carriers of heterologous antigens to induce immunity against a variety of infectious diseases. However, the amount of heterologous antigen required to induce an adequate immune response may be toxic for the bacterium and result in cell death, overattenuation or loss of expression of the heterologous antigen. To solve this problem an expression vector was developed with a strong promoter located on a DNA fragment which is inverted at random. Antigen is only expressed in one particular orientation of the promoter. Thus a bacterial population harbouring the plasmid will consist of a subpopulation which does not produce heterologous antigen, and is therefore not affected in growth, persistence and dissemination within the host. Further, this non-producing population will continuously segregate antigen-producing bacteria. To evaluate the system, **CtxB** was used as a model antigen. Analysis of the plasmid DNA isolated from Salmonella revealed a selection against the promoter orientation that directs transcription of the **ctxB** gene. In spite of this, the vector was stably maintained in vivo and induced **CtxB**-specific IgA and IgG in mice. These results indicate that this kind of expression vector may offer a solution to the problem of unstable expression of foreign antigens in live bacterial vaccine strains.

L20 ANSWER 85 OF 128 MEDLINE

95128188 Document Number: 95128188. PubMed ID: 7827509. Recombinant cholera toxin B subunit in Escherichia coli: high-level secretion,

purification, and characterization. Slos P; Speck D; Accart N; Kolbe H V; Schubnel D; Bouchon B; Bischoff R; Kieny M P. (Department of Bacterial Vectors, TRANSGENE S. A., Strasbourg, France.) PROTEIN EXPRESSION AND PURIFICATION, (1994 Oct) 5 (5) 518-26. Journal code: 9101496. ISSN: 1046-5928. Pub. country: United States. Language: English.

AB The gene coding for cholera toxin subunit B (CT-B) was fused to a modified ompA signal sequence and subsequently cloned into a high expression vector based on the regulatory signals of the arabinose operon of Salmonella typhimurium. Upon induction of gene expression in Escherichia coli, a product of the expected size for CT-B monomer was detected at a level of approximately 60% of total periplasmic protein. At pilot scale, batch cultivation in a 20-liter bioreactor allowed a production level of 1 g/liter of recombinant CT-B (rCT-B), the majority of which was released into the culture medium. The latter phenomenon was dependent on the medium selected for cultivation. A simple and inexpensive purification scheme was developed which enabled the recovery of 81% of rCT-B from the culture supernatant. Comparing amino acid composition, amino-terminal sequence, mass spectrum, pentamerisation, and GM1-binding, rCT-B is indistinguishable from natural CT-B produced by Vibrio cholerae. This rCT-B overproducing E. coli strain represents an interesting alternative to overexpressing systems developed in V. cholerae.

L20 ANSWER 86 OF 128 MEDLINE

94335720 Document Number: 94335720. PubMed ID: 8057923. Regulation of cholera toxin by temperature, pH, and osmolarity. Gardel C L; Mekalanos J J. (Department of Microbiology and Molecular Genetics, Harvard Medical School, Boston, Massachusetts 02115.) METHODS IN ENZYMOLOGY, (1994) 235 517-26. Ref: 40. Journal code: 0212271. ISSN: 0076-6879. Pub. country: United States. Language: English.

L20 ANSWER 87 OF 128 MEDLINE DUPLICATE 46

95234358 Document Number: 95234358. PubMed ID: 7718277. The upstream sequence of cholera toxin B subunit gene: effect on CTB expression. Cao C; Shi C; Li P; Ma Q. (Institute of Biotechnology, Academy of Military Medical Sciences, Beijing.) I CHUAN HSUEH PAO. ACTA GENETICA SINICA, (1994) 21 (6) 479-85. Journal code: 7900784. ISSN: 0379-4172. Pub. country: China. Language: Chinese.

AB In this work, we have studied the effect of cholera toxin A structure gene on the expression of the distal **ctxB** gene by the methods of deletion and frame-shift mutation. The results showed that: The expression level of Plasmid pUC19CTB, which was constructed by cloning the XbaI-EcoRI restriction fragment into pUC19 and ctxA gene was out-frame with lacZ' gene, is about 30 micrograms/ml; If a frame shift mutation was introduced at XbaI site of pUC19CTB so that the cholera toxin A gene was inframe with lacZ' and could be translated, the expression level of **ctxB** was decreased to 12 micrograms/ml; When A further deletion from XbaI to ClaI of cholera toxin A gene (about 550bp) was made and ctxA was outframe with LacZ', **ctxB** expression was decreased two fold compared to pUC19CTB; If the ctxA was inframe with LacZ' so ctxA gene could be translated, the expression level of CTB is much lower than the plasmid outframe with lacZ'. These observations could not be explained by the current knowledge about genetical regulation of cholera toxin operon. The promoter we found located in the cholera toxin A subunit gene, which is responsible for the expression of cholera toxin B subunit, may answer the question why the 550bp non-coding sequence could enhance the expression of cholera toxin B subunit.

L20 ANSWER 88 OF 128 MEDLINE

95215656 Document Number: 95215656. PubMed ID: 7701140. [Polymerase chain reaction (PCR) for the identification of toxigenic Vibrio cholerae O1 in oysters]. La tecnica de reaccion de polimerizacion en cadena (PCR) para la identificacion de Vibrio cholerae O1 toxigenico en ostiones. Rodriguez-Angeles M G; Giono-Cerezo S; Moreno-Escobar A; Valdespino-Gomez

J L. (Instituto Nacional de Diagnostico y Referencia Epidemiologicos, Mexico, D.F., Mexico.) REVISTA LATINOAMERICANA DE MICROBIOLOGIA, (1994 Oct-Dec) 36 (4) 295-306. Journal code: 0242625. ISSN: 0187-4640. Pub. country: Mexico. Language: Spanish.

AB PCR was made with ctx2 (CGG GCA GAT TCT AGA CCT CCT G) y ctx3 (CGA TGA TCT TGG AGC ATT CCC AC) primers for subunit A of cholera toxin, 30 cycles of temperature on samples of 50 g of oysters added in 450 ml of peptone alcaline water that were inoculated with 15 x 10(6), 0.75 x 10(6) and 0.15 x 10(6) CFU/ml of toxigenic 6707 V. cholerae O1 reference strain. The samples were tested by three microbiological methods: INDRE's method uses 1 x 10(-1) dilution of sample, two fold pass to peptone alcaline water pH 9 incubated 18 h and 6 h at 37 degrees C, the Food and Drugs Administration (FDA) method uses 10(-1) to 10(-6) dilutions of sample, 6 h incubation and reincubation for 18 h at 37 and 42 degrees C and the Mexican laboratories (LMD) with 10(-4) to 10(-3) dilutions, the samples were incubated for 6 h and then reincubated for 18 h at two temperatures 37 and 42 degrees C. The PCR by INDRE's method was positive with 3 x 10(2) CFU/ml/g oyster. In the FDA's method the PCR detected DNA in 10(-4) dilution with 3 x 10(1) CFU/ml/g oyster and in LMD's method the PCR was positive in 10(-3) with 3 CFU/ml/g oyster. The results of the PCR were obtained between 5-6 h, and later V. cholerae O1 was isolated by three microbiological methods. The PCR reproducibility was better on DNA sample diluted 1:4 and 10 microliters of sample increased from 1:1000 to 1:10000 the sensitivity of PCR.

L20 ANSWER 89 OF 128 MEDLINE

95215652 Document Number: 95215652. PubMed ID: 7701136. [Cytotonic and cytotoxic effect of cholera toxin on Vero cells and its relation to PCR]. Efecto citotonico y citotoxico de la toxina colerica en celulas Vero y su relacion con PCR. Rodriguez-Angeles M G; Giono-Cerezo S; Valdespino-Gomez J L. (Instituto Nacional de Diagnostico y Referencia Epidemiologicos, Mexico, D.F., Mexico.) REVISTA LATINOAMERICANA DE MICROBIOLOGIA, (1994 Oct-Dec) 36 (4) 263-71. Journal code: 0242625. ISSN: 0187-4640. Pub. country: Mexico. Language: Spanish.

AB We studied 40 Vibrio cholerae strains: 16 from stool, 16 from sewage and 8 from food. The serotypes were Inaba in 21 strains, 8 Ogawa strains and 11 V. cholerae non-O1. PCR was made with ctx2 and ctx3 primers with 25 cycles of temperature: 1 min at 94 degrees C, 1 min at 60 degrees C and 1 min at 72 degrees C. 24 V. cholerae strains were positive: 18/24 Inaba y 6/24 Ogawa. PCR was negative for 16 strains: 3 Inaba serotype, 2 Ogawa y 11 V. cholerae non-O1. In Vero culture cells 18 strains were cytotonic, 21 cytotoxic and 1 strain was negative. ELISA was positive for 11 strains with PCR positive. The PCR sensitivity was 95.83% compared with culture cells. V. cholerae O1 produced cytotoxic effect on Vero culture cells, maybe related to ACE factor. Colony blot was made with a specific probe labeled with digoxigenin and it could detect 4 Vibrio cholerae toxigenic strains with PCR negative. All V. cholerae Non O1 strains were PCR negative.

L20 ANSWER 90 OF 128 MEDLINE

95215649 Document Number: 95215649. PubMed ID: 7701133. [Phenotypic and genotypic characterization of Vibrio cholerae O1]. Caracterization fenotipica y genotipica de Vibrio cholerae O1. Giono-Cerezo S; Rodriguez Angeles M G; Gutierrez-Cogco L; Valdespino-Gomez J L. (Laboratorio de Bacteriologia Enterica, Instituto Nacional de Diagnostico y Referencia Epidemiologicos, Mexico, D.F., Mexico.) REVISTA LATINOAMERICANA DE MICROBIOLOGIA, (1994 Oct-Dec) 36 (4) 243-51. Journal code: 0242625. ISSN: 0187-4640. Pub. country: Mexico. Language: Spanish.

AB We made 52180 tests for isolation and identification of toxigenic V. cholerae O1 from rectal swabs and reference strains. We isolated 17.6% V. cholerae O1 strains in 1991, 43.5% in 1992 and 38.9% in 1993. The main serovar in 1991 was Inaba, whereas in 1993 a similar percentage was serovar Ogawa. The phenotype of V. cholerae strains was determined by

hemolysis test, Voges-Proskauer test, polymyxin B resistance and phages 4 and 5 resistance. All of the mexican strains were El Tor. There were 2.9-0.75% hemolytic strains from 1991 to 1993, but they were negative when the test was made in tube with human erythrocytes. The resistotypes were performed in 24526 selected strains by Kirby-Bauer method and MIC tests. All of the strains were sensitive, except more than 100 strains isolated in Veracruz that were resistant to tetracycline and doxycycline. Detection of cholera toxin was made by ELISA and on culture of Vero and CHO cells. All the V. cholerae O1 strains were toxigenic. The genotype was determined by PCR and ribotyping. The PCR amplified one 564 pb fragment on V. cholerae O1. The ribotypes of mexican strains were 5 and 6a.

L20 ANSWER 91 OF 128 MEDLINE DUPLICATE 47

94237453 Document Number: 94237453. PubMed ID: 8181723. Structure and arrangement of the cholera toxin genes in Vibrio cholerae O139. Lebens M; Holmgren J. (University of Gothenburg, Department of Medical Microbiology and Immunology, Goteborg, Sweden.) FEMS MICROBIOLOGY LETTERS, (1994 Apr 1) 117 (2) 197-202. Journal code: 7705721. ISSN: 0378-1097. Pub. country: Netherlands. Language: English.

AB The sequence of the **ctxB** gene encoding the B subunit of cholera toxin has been determined for a strain of Vibrio cholerae of the novel O139 serotype associated with recent outbreaks of severe cholera throughout South-East Asia and found to be identical to the **ctxB** gene in V. cholerae O1 of the El Tor biotype. Analyses by Southern hybridization and PCR showed that all strains of the O139 serotype V. cholerae tested carried cholera toxin genes and other genes associated with a virulence cassette DNA region at two loci identical or homologous to those identified in the Classical rather than the El Tor biotype of V. cholerae serotype O1 although these loci in O139 could reside on restriction fragments of variable size.

L20 ANSWER 92 OF 128 CAPLUS COPYRIGHT 2002 ACS

1993:464935 Document No. 119:64935 Membrane targeting of heterologous proteins in bacteria and use of the bacteria or membranes as vaccines. Niesel, David W.; Moncrief, J. Scott; Phillips, Linda H. (University of Texas System, USA). PCT Int. Appl. WO 9310246 A1 19930527, 73 pp. DESIGNATED STATES: W: AT, AU, BB, BG, BR, CA, CH, CS, DE, DK, ES, FI, GB, HU, JP, KP, KR, LK, LU, MG, MN, MW, NL, NO, PL, RO, RU, SD, SE, UA; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, DE, DK, ES, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NL, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1992-US9659 19921112. PRIORITY: US 1991-792252 19911115.

AB A method for producing heterologous proteins, esp. antigens, in bacterial cells and exporting them to the inner membrane/periplasm or the outer membrane surface is described. Bacterial cells, or membranes of these cells, contg. these heterologous proteins may be used as vaccines. Plasmids pZIP-IN and pZIP-OUT, which contain protein-encoding Salmonella typhimurium genomic DNA fused to the Escherichia coli phoA gene, direct the fusion protein to the inner and outer membranes, resp. A pZIP-OUT deriv., in which a **ctxB** gene fragment was fused to the phoA sequence, was prepd. The chimeric gene, encoding a tripartite S. typhimurium protein-PhoA-**CtxB** fusion, was expressed in an attenuated Salmonella strain. The cholera toxin B subunit peptide was immunol. localized to the outer surface membrane.

L20 ANSWER 93 OF 128 MEDLINE DUPLICATE 48

94011341 Document Number: 94011341. PubMed ID: 8406837. CVD110, an attenuated Vibrio cholerae O1 El Tor live oral vaccine strain. Michalski J; Galen J E; Fasano A; Kaper J B. (Department of Medicine, University of Maryland School of Medicine, Baltimore 21201.) INFECTION AND IMMUNITY, (1993 Oct) 61 (10) 4462-8. Journal code: 0246127. ISSN: 0019-9567. Pub. country: United States. Language: English.

AB The recent expansion of the seventh cholera pandemic into South America emphasizes the need for a safe, long-lasting, protective, and

nonreactogenic vaccine for this disease. Since the predominant Vibrio cholerae O1 strains in the world today are of the El Tor biotype, a bivalent vaccine containing both classical and El Tor biotypes may be desirable. We have constructed a new oral vaccine candidate, V. cholerae CVD110 El Tor, Ogawa, from which all toxin genes so far identified in V. cholerae have been deleted. Three of these genes, those encoding cholera toxin (ctx), zonula occludens toxin (zot), and accessory cholera enterotoxin (ace), are located on a 4.5-kb virulence cassette flanked by repetitive sequences (RS1 elements). Homologous recombination between these RS1 elements resulted in the deletion of this virulence cassette to yield V. cholerae CVD109. Insertion of genes encoding mercury resistance (mer) and the cholera toxin B subunit (**ctxB**) into the hemolysin locus (hlyA) produced CVD110. This insertion serves three purpose. (i) It genetically tags the vaccine strain so as to distinguish it from wild-type V. cholerae O1. (ii) It produces cholera toxin B subunit in order to elicit antitoxic immunity. (iii) It inactivates the hemolysin gene, rendering the strain nonhemolytic on sheep erythrocyte plates. Supernatants from V. cholerae CVD110 cultures are nonreactogenic when assayed in Ussing chambers.

L20 ANSWER 94 OF 128 MEDLINE

93234575 Document Number: 93234575. PubMed ID: 8475125. CTX genetic element encodes a site-specific recombination system and an intestinal colonization factor. Pearson G D; Woods A; Chiang S L; Mekalanos J J. (Department of Microbiology and Molecular Genetics, Harvard Medical School, Boston, MA 02115.) PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (1993 Apr 15) 90 (8) 3750-4. Journal code: 7505876. ISSN: 0027-8424. Pub. country: United States. Language: English.

AB In Vibrio cholerae, the genes encoding cholera toxin (ctxAB) are located on a segment of DNA (termed the "core" region) that is flanked by two or more copies of a repeated sequence called RS1. Together these DNA units comprise the CTX genetic element. Evidence presented here suggests that RS1 sequences encode a site-specific recombination system, which allows integration of a suicide plasmid carrying RS1 into an 18-base-pair sequence (attRS1) located on the chromosome of nontoxigenic V. cholerae strains. Strains of V. cholerae with large deletions removing attRS1 and the entire CTX genetic element no longer undergo site-specific recombination with the RS1 sequence. Additionally, these deletion strains show a defect in intestinal colonization. Recombination experiments localize the gene responsible for enhancing colonization to a portion of the core region of the CTX element. The identified gene encodes a peptide that is highly similar in amino acid sequence to the flexible pilin of Aeromonas hydrophila. These results have important implications in the construction of stable, live attenuated cholera vaccines.

L20 ANSWER 95 OF 128 MEDLINE

94059512 Document Number: 94059512. PubMed ID: 7764248. Large-scale production of Vibrio cholerae toxin B subunit for use in oral vaccines. Lebens M; Johansson S; Osek J; Lindblad M; Holmgren J. (University of Goteborg, Dept. of Medical Microbiology and Immunology, Sweden.) BIO/TECHNOLOGY, (1993 Dec) 11 (13) 1574-8. Journal code: 8309273. ISSN: 0733-222X. Pub. country: United States. Language: English.

AB By systematically manipulating promoter and ribosome binding structures, plasmid copy number and the structure of the cholera toxin B (CTB) subunit gene, we were able to develop a plasmid expression system that, when used in conjunction with an optimized growth medium, provided yields of CTB approaching one gram per liter. The CTB protein which was secreted to > 95%, could readily be purified from the growth medium of a V. cholerae production strain and was shown to be immunologically indistinguishable from previously used vaccine preparations of native or recombinant CTB.

L20 ANSWER 96 OF 128 MEDLINE DUPLICATE 49

93179785 Document Number: 93179785. PubMed ID: 7680060. The molecular epidemiology of cholera in Latin America. Wachsmuth I K; Evins G M; Fields P I; Olsvik O; Popovic T; Bopp C A; Wells J G; Carrillo C; Blake P A. (Enteric Diseases Branch, Centers for Disease Control, Atlanta, Georgia; Atlanta, GA 30333.) JOURNAL OF INFECTIOUS DISEASES, (1993 Mar) 167 (3) 621-6. Journal code: 0413675. ISSN: 0022-1899. Pub. country: United States. Language: English.

AB To explain the sudden appearance and rapid spread of cholera in Latin America in January 1991, molecular techniques were used to define Vibrio cholerae O1 isolates from around the world. Restriction fragment length polymorphisms of rRNA and ctxA genes, DNA sequence of cholera toxin B subunit gene **ctxB**, and multilocus enzyme electrophoresis data were used to characterize 197 isolates. Worldwide, there are at least four distinct toxigenic El Tor V. cholerae O1 clones: the seventh pandemic (Eastern Hemisphere), US Gulf Coast, Australian, and Latin American. Nontoxigenic V. cholerae O1 previously isolated in Brazil, Mexico, and Peru are unlike current toxigenic isolates. The Latin American clone probably represents an extension of the seventh pandemic into the Western Hemisphere, while the US Gulf Coast clone most likely evolved separately. These data will be useful in monitoring the spread of cholera, determining the origin of outbreaks in both hemispheres, and implicating specific vehicles of transmission.

L20 ANSWER 97 OF 128 MEDLINE DUPLICATE 50

93138755 Document Number: 93138755. PubMed ID: 8423068. Reduction in oral immunogenicity of cholera toxin B subunit by N-terminal peptide addition. Dertzbaugh M T; Elson C O. (Division of Gastroenterology, School of Medicine, University of Alabama, Birmingham 35294.) INFECTION AND IMMUNITY, (1993 Feb) 61 (2) 384-90. Journal code: 0246127. ISSN: 0019-9567. Pub. country: United States. Language: English.

AB The mucosal adjuvanticity of cholera toxin and the potential of the B subunit of cholera toxin (**CtxB**) to serve as an oral vaccine carrier have prompted interest in the coupling of immunogenic peptides to this protein. The purpose of this study was to determine how such fusions affect the function of **CtxB**. Oligonucleotides were genetically fused to the 5' terminus of the **ctxB** gene to encode additional amino acids of 8, 12, and 24 residues in length. None of these additions affected the ability of **CtxB** to oligomerize, as determined by nondenaturing sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Circular dichroism revealed no difference in conformation between the modified B subunits, regardless of the length of the addition. However, when compared with native **CtxB**, additions to the N terminus induced a consistent change in the net conformation of the protein. By using a competitive enzyme immunoassay, the affinity of the modified B subunits for GM1 ganglioside was shown to gradually decrease with increasing length of the N-terminal addition. A similar pattern was observed for the ability of the chimeras to inhibit proliferation of concanavalin A-stimulated spleen cells in vitro, which is a previously described functional property of **CtxB** that is dependent on its binding to cells. Lastly, the oral immunogenicity of these chimeras was found to be less than that of native **CtxB**. These results indicate that large fusions to the N terminus of **CtxB** can significantly affect its biological properties and could reduce its value as a mechanism for effective mucosal immunization.

L20 ANSWER 98 OF 128 MEDLINE

93175126 Document Number: 93175126. PubMed ID: 7679865. Current progress in the development of the B subunits of cholera toxin and Escherichia coli heat-labile enterotoxin as carriers for the oral delivery of heterologous antigens and epitopes. Nashar T O; Amin T; Marcello A; Hirst T R. (Biological Laboratory, University of Kent, Canterbury, UK.) VACCINE, (1993) 11 (2) 235-40. Ref: 49. Journal code: 8406899. ISSN: 0264-410X. Pub. country: ENGLAND: United Kingdom. Language: English.

AB The development of non-living carrier systems for delivery of protective antigens or epitopes to the immune system represents both a fundamental and an applied aspect of vaccinology. A wide range of carrier systems, ranging from inert supports to proteins that exert direct immunomodulating effects on the immune response, are being studied. In this overview we describe the current progress in the development of the B-subunits of cholera toxin and Escherichia coli heat-labile enterotoxin as potential protein carriers for the oral delivery of chemically and genetically attached antigens and epitopes.

L20 ANSWER 99 OF 128 MEDLINE

94040815 Document Number: 94040815. PubMed ID: 7693553. Immunological characterization of a rotavirus-neutralizing epitope fused to the cholera toxin B subunit. Gonzalez R A; Sanchez J; Holmgren J; Lopez S; Arias C F. (Departamento de Biologia Molecular, Universidad Nacional Autonoma de Mexico, Cuernavaca, Mexico.) GENE, (1993 Nov 15) 133 (2) 227-32. Journal code: 7706761. ISSN: 0378-1119. Pub. country: Netherlands. Language: English.

AB A highly conserved neutralizing epitope from the surface protein VP4 (amino acids 296-313) of human rotaviruses was genetically fused to the B subunit of cholera toxin (CTB). Synthetic oligodeoxyribonucleotides encoding the VP4 peptide were inserted between the 3' end of the DNA that codes for the leader peptide, and the 5' end of the gene encoding mature CTB. The hybrid protein synthesized in Escherichia coli was found to maintain the ability of CTB to pentamerize, and to adhere to its cell receptor, the GM1 ganglioside. The chimera was efficiently recognized by a monoclonal antibody (mAb) directed at CTB and by a virus-neutralizing mAb against the VP4 peptide. The hybrid polypeptide was shown to induce high titers of serum antibodies (Ab) against CTB and the synthetic VP4 peptide following subcutaneous immunization; paradoxically, however, the Ab obtained did not recognize the virus by an enzyme-linked immunosorbent assay method, nor had detectable neutralizing activity. Potential implications of these results for future design and evaluation of fusion proteins as immunogens are discussed.

L20 ANSWER 100 OF 128 MEDLINE DUPLICATE 51

93114904 Document Number: 93114904. PubMed ID: 8418065. Comparative effectiveness of the cholera toxin B subunit and alkaline phosphatase as carriers for oral vaccines. Dertzbaugh M T; Elson C O. (Division of Gastroenterology, School of Medicine, University of Alabama, Birmingham 35294.) INFECTION AND IMMUNITY, (1993 Jan) 61 (1) 48-55. Journal code: 0246127. ISSN: 0019-9567. Pub. country: United States. Language: English.

AB The purpose of this study was to determine whether the B subunit of cholera toxin (**CtxB**) has adjuvant activity over and above serving as a carrier protein for orally administered vaccines. An oligonucleotide that encodes an antigenic determinant (GtfB.1) from the glucosyltransferase B gene (gtfB) of Streptococcus mutans was genetically fused to the 5' terminus of either the **CtxB** gene (**ctxB**) or the Escherichia coli alkaline phosphatase gene (phoA). The resulting chimeric proteins were expressed in a phoA mutant strain of E. coli and then purified. The antigenicities of the proteins were confirmed by immunoblotting analysis using antisera specific for GtfB, **CtxB**, or PhoA. An equimolar amount of peptide on each carrier was administered by gastric intubation to mice three times at 10-day intervals. Antibody titers to the peptide, **CtxB**, and PhoA (in the serum, intestine, vagina, saliva, and bronchus) were determined by enzyme immunoassay. Antibody to the peptide was detected only in the sera of mice immunized with the peptide fused to **CtxB**. No antipeptide antibody was detected in mice immunized with the peptide fused to PhoA. The lack of detectable levels of antipeptide antibody in intestinal lavage fluid was attributed to dilution of the sample beyond the sensitivity of the assay. This was confirmed by cultivation of Peyer's patch and mesenteric lymph node tissue from mice orally immunized with the GtfB.1::**CtxB**

chimera. Using this method, antipeptide antibody was detected in the culture fluid. We conclude that **CtxB** possesses unique properties that allow it to act as more than a simple carrier protein.

L20 ANSWER 101 OF 128 MEDLINE DUPLICATE 52
93107263 Document Number: 93107263. PubMed ID: 7678018. Use of automated sequencing of polymerase chain reaction-generated amplicons to identify three types of cholera toxin subunit B in Vibrio cholerae O1 strains. Olsvik O; Wahlberg J; Petterson B; Uhlen M; Popovic T; Wachsmuth I K; Fields P I. (Division of Bacterial and Mycotic Diseases, Centers for Disease Control, Atlanta, Georgia 30333.) JOURNAL OF CLINICAL MICROBIOLOGY, (1993 Jan) 31 (1) 22-5. Journal code: 7505564. ISSN: 0095-1137. Pub. country: United States. Language: English.

AB Cholera toxin is the principal factor causing the profuse intestinal fluid secretion that is characteristic of cholera. The DNA sequences of the cholera toxin subunit B structural genes from 45 Vibrio cholerae O1 strains isolated in 29 countries over a period of 70 years were determined by automated DNA sequencing of polymerase chain reaction-generated amplicons. Three types of cholera toxin B subunit gene (**ctxB**) were identified. Genotype 1 was found in strains of classical biotype worldwide and El Tor biotype strains associated with the U.S. Gulf Coast, genotype 2 was found in El Tor biotype strains from Australia, and genotype 3 was found in El Tor biotype strains from the seventh pandemic and the recent Latin American epidemic. All base changes correspond to an amino acid substitution in the B subunit of the cholera toxin. Heterogenicity in the B subunit could have implications for vaccine development and diagnostic tests for cholera toxin and antitoxin. We conclude that this technology provides timely and potentially useful epidemiological information.

L20 ANSWER 102 OF 128 CAPLUS COPYRIGHT 2002 ACS
1993:58102 Document No. 118:58102 Preparation of immunoprotective antigens of pneumococcal surface protein A. Briles, David E.; Yother, Janet L.; McDaniel, Larry S. (UAB Research Foundation, USA). PCT Int. Appl. WO 9214488 A1 19920903, 44 pp. DESIGNATED STATES: W: AU, CA, FI, JP, NO, US; RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LU, MC, NL, SE. (English). CODEN: PIXXD2. APPLICATION: WO 1992-US857 19920212. PRIORITY: US 1991-656773 19910215.

AB A purified, truncated pneumococcal surface protein A (PspA) presenting epitopes is prepd. with a mutant of Streptococcus pneumoniae for use in vaccines. This antigen is useful because it is independent of pneumococcal capsular type. The truncated PspA lacks at least the functional cell membrane anchor domain, or the repeat region and the proline-rich regions. A 43-kDa (apparent mol. wt.) truncated PspA was secreted by S. pneumoniae Rx1, a novel mutant prepd. by insertional duplication mutagenesis. This truncated PspA gene can be expressed in Mycobacterium or Escherichia coli, e.g. as a fusion protein with the B-unit of cholera toxin (CTB), for prepn. of vaccine against pneumococcal infection.

L20 ANSWER 103 OF 128 MEDLINE
93014215 Document Number: 93014215. PubMed ID: 1399002. Fusion proteins containing the A2 domain of cholera toxin assemble with B polypeptides of cholera toxin to form immunoreactive and functional holotoxin-like chimeras. Jobling M G; Holmes R K. (Department of Microbiology, Uniformed Services University of the Health Sciences, Bethesda, Maryland 20814.) INFECTION AND IMMUNITY, (1992 Nov) 60 (11) 4915-24. Journal code: 0246127. ISSN: 0019-9567. Pub. country: United States. Language: English.

AB Cholera enterotoxin (CT) is produced by Vibrio cholerae and excreted into the culture medium as an extracellular protein. CT consists of one A polypeptide and five B polypeptides associated by noncovalent bonds, and CT-B interacts with CT-A primarily via the A2 domain. Treatment of CT with trypsin cleaves CT-A into A1 and A2 fragments that are linked by a

disulfide bond. CT-B binds to ganglioside GM1, which functions as the plasma membrane receptor for CT, and the enzymatic activity of A1 causes the toxic effects of CT on target cells. We constructed translational fusions that joined foreign proteins via their carboxyl termini to the A2 domain of CT-A, and we studied the interactions of the fusion proteins with CT-B. The A2 domain was necessary and sufficient to enable bacterial alkaline phosphatase (BAP), maltose-binding protein (MBP) or beta-lactamase (BLA) to associate with CT-B to form stable, immunoreactive, holotoxin-like chimeras. Each holotoxin-like chimera was able to bind to ganglioside GM1. Holotoxin-like chimeras containing the BAP-A2 and BLA-A2 fusion proteins had BAP activity and BLA activity, respectively. We constructed BAP-A2 mutants with altered carboxyl-terminal sequences and tested their ability to assemble into holotoxin-like chimeras. Although the carboxyl-terminal QDEL sequence of the BAP-A2 fusion protein was not required for interaction with CT-B, most BAP-A2 mutants with altered carboxyl termini did not form holotoxin-like chimeras. When holotoxin-like chimeras containing BAP-A2, MBP-A2, or BLA-A2 were synthesized in V. cholerae, they were found predominantly in the periplasm. The toxin secretory apparatus of V. cholerae was not able, therefore, to translocate these holotoxin-like chimeras across the outer membrane.

L20 ANSWER 104 OF 128 MEDLINE DUPLICATE 53
92289698 Document Number: 92289698. PubMed ID: 1600950. Selective extracellular release of cholera toxin B subunit by Escherichia coli: dissection of Neisseria Iga beta-mediated outer membrane transport. Klauser T; Pohlner J; Meyer T F. (Max-Planck-Institut fur Biologie, Abteilung Infektionsbiologie, Tubingen, FRG.) EMBO JOURNAL, (1992 Jun) 11 (6) 2327-35. Journal code: 8208664. ISSN: 0261-4189. Pub. country: ENGLAND: United Kingdom. Language: English.

AB The C-terminal domain (Iga beta) of the Neisseria IgA protease precursor is involved in the transport of covalently attached proteins across the outer membrane of Gram-negative bacteria. We investigated outer membrane transport in Escherichia coli using fusion proteins consisting of an N-terminal signal sequence for inner membrane transport, the Vibrio cholerae toxin B subunit (**CtxB**) as a passenger and Iga beta. The process probably involves two distinct steps: (i) integration of Iga beta into the outer membrane and (ii) translocation of the passenger across the membrane. The outer membrane integrated part of Iga beta is the C-terminal 30 kDa core, which serves as a translocator for both the passenger and the linking region situated between the passenger and Iga beta core. The completeness of the translocation is demonstrated by the extracellular release of the passenger protein owing to the action of the E. coli outer membrane OmpT protease. Translocation of the **CtxB** moiety occurs efficiently under conditions preventing intramolecular disulphide bond formation. In contrast, if disulphide bond formation in the periplasm proceeds, then translocation halts after the export of the linking region. In this situation transmembrane intermediates are generated which give rise to characteristic fragments resulting from rapid proteolytic degradation of the periplasmically trapped portion. Based on the identification of translocation intermediates we propose that the polypeptide chain of the passenger passes in a linear fashion across the bacterial outer membrane.

L20 ANSWER 105 OF 128 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
1992:270440 Document No.: BR42:129390. COMPARISON OF THE ORAL IMMUNOGENICITY OF A FOREIGN PEPTIDE WHEN COUPLED TO CHOLERA TOXIN B SUBUNIT OR TO ALKALINE PHOSPHATASE. DERTZBZUGH M T; ELSON C O. UNIV. ALABAMA BIRMINGHAM, BIRMINGHAM, ALA. 35294.. MEETING OF THE FEDERATION OF AMERICAN SOCIETIES FOR EXPERIMENTAL BIOLOGY (FASEB), PART 1, ANAHEIM, CALIFORNIA, USA, APRIL 5-9, 1992. FASEB (FED AM SOC EXP BIOL) J. (1992) 6 (4), A1229. CODEN: FAJOEC. ISSN: 0892-6638. Language: English.

L20 ANSWER 106 OF 128 MEDLINE

93110970 Document Number: 93110970. PubMed ID: 1361701. Molecular design of cholera vaccines. Manning P A. (Department of Microbiology and Immunology, University of Adelaide, South Australia.) VACCINE, (1992) 10 (14) 1015-21. Ref: 64. Journal code: 8406899. ISSN: 0264-410X. Pub. country: ENGLAND: United Kingdom. Language: English.

AB Cholera is still a serious public health problem in developing countries, particularly those in tropical regions. This has stimulated considerable research into the molecular analysis of pathogenesis resulting in the identification of a number of critical components required for both colonization of the gut mucosa and the disease symptoms. These components are the targets for rational molecular approaches to vaccine development.

L20 ANSWER 107 OF 128 MEDLINE DUPLICATE 54

92339828 Document Number: 92339828. PubMed ID: 1634070. Oral immunization against cholera toxin with a live Yersinia enterocolitica carrier in mice. Van Damme M; Sory M P; Biot T; Vaerman J P; Cornelis G R. (Experimental Medicine Unit, Universite Catholique de Louvain, Brussels, Belgium.) GASTROENTEROLOGY, (1992 Aug) 103 (2) 520-31. Journal code: 0374630. ISSN: 0016-5085. Pub. country: United States. Language: English.

AB The 70-kb pYV plasmid of Yersinia enterocolitica directs the synthesis and secretion of several virulence determinants called Yops. These proteins are produced during the invasion of the host tissues and induce a strong antibody response. The yop genes are transcribed from strong promoters activated by a common transcription activator. Recombinant Y. enterocolitica strains expressing the B subunit of the cholera toxin were constructed from a yopH-**ctxB** operon fusion. Integration of the gene **ctxB** in the pYV plasmid itself, by a double crossing over, ensured its stability in the infecting bacteria. Oral inoculation of recombinant bacteria in mice elicited serum and intestinal antibody responses and resulted in protection of the immunized mice against a cholera toxin challenge. Secretory immunoglobulin A antibodies against the cholera toxin B subunit occurred not only in the intestines but also in the respiratory tract.

L20 ANSWER 108 OF 128 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.

1992:400616 Document No.: BR43:56491. THREE GENOTYPES OF VIBRIO-CHOLERAE O1 ENTEROTOXIN BASED ON DNA SEQUENCES. OLSVIK O; FIELDS P; WACHSMUTH I K; WAHLBERG J; PETTERSON B; UHLEN M. CENT. DISEASE CONTROL, ATLANTA, GA.. 92ND GENERAL MEETING OF THE AMERICAN SOCIETY FOR MICROBIOLOGY, NEW ORLEANS, LOUISIANA, USA, MAY 26-30, 1992. ABSTR GEN MEET AM SOC MICROBIOL. (1992) 92 (0), 453. CODEN: AGMME8. Language: English.

L20 ANSWER 109 OF 128 CAPLUS COPYRIGHT 2002 ACS

1992:627316 Document No. 117:227316 Plasmids that allow the continuous expression of antigens in Salmonella typhimurium at toxic levels. Tijhaar, Edwin J.; Karlas, Jos A.; Van Els, Cecile A. C. M.; Bosch, Marnix L.; Osterhaus, Albert D. M. E.; Mool, Frits R.; Zhengxin, Yan; Meyer, Thomas F. (Dep. Immunobiol., Natl. Inst. Public Health Enviorn. Prot., Bilthoven, 3720 BA, Neth.). Vaccines 92: Mod. Approaches New Vaccines Incl. Prev. AIDS [Annu. Meet.], 9th, 379-84. Editor(s): Brown, Fred. Cold Spring Harbor Lab. Press: Cold Spring Harbor, N. Y. (English) 1992. CODEN: 57WXAL.

AB Plasmids, contg. an invertible PL promoter, that allow the continuous expression of antigens at a toxic level in a bacterial population are described. Using **CtxB** as a model antigen, S. typhimurium aroA strains carrying one of these plasmids (pYZ17) are shown to be able to induce **CtxB**-specific IgG and IgA responses after oral administration to mice. Furthermore, it appeared that pYZ17 was stably maintained in vivo. Expression of **CtxB** could be increased considerably by constructing a two-plasmid system in which the antigen of interest was expressed by T7 RNA polymerase, the prodn. of which is controlled by an invertible promoter. The two-plasmid system was used to

express an HIV-1-env fusion protein. Although large amts. of the env fusion protein were produced by S. typhimurium aroA, the plasmids were stably maintained in vitro. The ability of S. typhimurium aroA carrying the two-plasmid system to induce a humoral and cellular immune response against the HIV-1 envelope protein is currently under investigation.

L20 ANSWER 110 OF 128 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC. DUPLICATE 55

1993:229034 Document No.: PREV199395120209. Vibrio cholerae toxin B subunit gene expressed in a Salmonella vaccine strain. Tian, Jinghui; Lu, Deru. Second Military Med. Univ., Shanghai 200433 China. Acta Microbiologica Sinica, (1992) Vol. 32, No. 5, pp. 320-327. Language: Chinese. Summary Language: Chinese; English.

AB This paper reports that the V. cholerae toxin B subunit (**ctxB**) gene was inserted into pYA248 plasmid with the aspartate beta-semialdehyde dehydrogenase (asd) gene and the recombinant plasmid was transformed into S. typhimurium deleting asd gene. Results showed that **ctxB** gene was highly expressed and secreted into medium. This strain was able to colonize in the intestinal epithelium. Oral immunity and general immunity could produce antibodies at high level and enhance cellular immune responses. The animals orally inoculated with S. typhimurium times 4072 (pYA-**ctxB**) vaccine had remarkable protection against virulent V. cholerae 569B strain and S. typhimurium strain. Use of such system provides useful method for oral vaccine.

L20 ANSWER 111 OF 128 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.

1992:381524 Document No.: BR43:48474. SALMONELLA EXPOSITION VECTORS DERIVED FROM TNPHOA FUSION STRAINS. PHILLIPS L; MONCRIEF S; NIESEL D. UNIV. TEXAS MED. BRANCH, GALVESTON, TEX.. 92ND GENERAL MEETING OF THE AMERICAN SOCIETY FOR MICROBIOLOGY, NEW ORLEANS, LOUISIANA, USA, MAY 26-30, 1992. ABSTR GEN MEET AM SOC MICROBIOL. (1992) 92 (0), 225. CODEN: AGMME8. Language: English.

L20 ANSWER 112 OF 128 MEDLINE DUPLICATE 56

93013965 Document Number: 93013965. PubMed ID: 1398801. Genetics of cholera toxin. Kaper J B; Srivastava B S. (Center for Vaccine Development, University of Maryland, Baltimore.) INDIAN JOURNAL OF MEDICAL RESEARCH, (1992 Jul) 95 163-7. Ref: 47. Journal code: 0374701. ISSN: 0971-5916. Pub. country: India. Language: English.

AB Cholera is caused by the toxin secreted by Vibrio cholerae 01. Cholera toxin (CT) is a protein consisting of A and B subunits. The former contributes to intracellular toxicity whereas the B subunit is required for binding of CT to eukaryotic cell surface receptor. The structural genes encoding A and B subunits are designated as ctxA and **ctxB** respectively. These genes are located on the chromosome forming an operon in which ctxA precedes **ctxB**. The ctxAB have been cloned and sequenced. Classical strains contain two full copies of unlinked ctxAB. Most el tors have single copy. However, in some strains there are two copies which are arranged in tandem. The tandem duplication and amplification of ctxAB is controlled by a transposable element like DNA sequence called RS1. A number of genes have been identified which regulate the expression of ctx operon. V. cholerae seems to elaborate more than one toxin which are different from the one encoded by ctxAB genes.

L20 ANSWER 113 OF 128 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.

1992:381109 Document No.: BR43:48059. CHOLERA TOXIN B-SUBUNIT EXPRESSION BY CTXA NEGATIVE VIBRIO-CHOLERAE O1 DELETION MUTANTS. FOXALL P A; SILVEIRA A P D; HALL R H. DIV. INFECT. DIS., UNIV. MD. SCH. MED., BALTIMORE, MD. 21201, USA.. 92ND GENERAL MEETING OF THE AMERICAN SOCIETY FOR MICROBIOLOGY, NEW ORLEANS, LOUISIANA, USA, MAY 26-30, 1992. ABSTR GEN MEET AM SOC MICROBIOL. (1992) 92 (0), 156. CODEN: AGMME8. Language: English.

L20 ANSWER 114 OF 128 CAPLUS COPYRIGHT 2002 ACS

1992:100630 Document No. 116:100630 Avirulent Vibrio cholerae strains and method for production of same by directed deletion of genomic material. Kaper, James B.; Baudry-Maurelli, Bernadette; Fasano, Alessio (University of Maryland, USA). PCT Int. Appl. WO 9118979 A1 19911212, 82 pp. DESIGNATED STATES: W: AU, CA, JP, SU; RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LU, NL, SE. (English). CODEN: PIXXD2. APPLICATION: WO 1991-US3812 19910605. PRIORITY: US 1990-533315 19900605.

AB V. cholerae of the Ogawa or Inaba serotype having deletions in the toxin and in the zonula occludens toxin genes are described. Methods for forcing directed deletions in Vibrio cholerae to cause attenuation of virulence without loss of ability to colonize the intestine are described. The resulting bacteria are suitable for use in live oral vaccines. The method uses a plasmid carrying a region derived from one of the virulence genes that is interrupted and a selectable marker. This is introduced into the virulent host by std. mating where it usually integrates into the host chromosome. The plasmid-carrying host is then mated with a strain carrying a second plasmid that is incompatible with the first. The mating is then selected for bacteria carrying the second selectable markers only; this will only be possible when the first marker has been excised from the chromosome by homologous recombination. The recombination also results in deletion of a region of the chromosome carried by the plasmid. Plasmids were constructed for use in the disruption of the genes for cholera toxin and zona occludens toxin and their uses is demonstrated.

L20 ANSWER 115 OF 128 CAPLUS COPYRIGHT 2002 ACS

1991:576713 Document No. 115:176713 Recombinant cholera toxin-antigenic peptide fusion proteins and their use as vaccines. Dertzbaugh, Mark T.; Macrina, Francis L. (Center for Innovative Technology, USA). PCT Int. Appl. WO 9107979 A1 19910613, 68 pp. DESIGNATED STATES: W: CA, JP; RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LU, NL, SE. (English). CODEN: PIXXD2. APPLICATION: WO 1990-US6811 19901128. PRIORITY: US 1989-442783 19891129.

AB Recombinant fusion proteins comprising an antigen epitope linked to the N-terminus of a cholera toxin B subunit fragment are produced. These may be used as vaccines. A chimeric gene contg. the ompA signal sequence, a fragment of the glucosyltransferase B gene (gtfB) of Streptococcus mutans, and a part of the **ctxB** gene was constructed and expressed in Escherichia coli. Antisera to the fusion protein produced by these transformants inhibited the S. mutans enzyme in vitro. This recombinant protein is proposed as a vaccine against dental caries.

L20 ANSWER 116 OF 128 MEDLINE

91210174 Document Number: 91210174. PubMed ID: 1902210. Expression of the Vibrio cholerae gene encoding aldehyde dehydrogenase is under control of ToxR, the cholera toxin transcriptional activator. Parsot C; Mekalanos J J. (Department of Microbiology and Molecular Genetics, Harvard Medical School, Boston, Massachusetts 02115.) JOURNAL OF BACTERIOLOGY, (1991 May) 173 (9) 2842-51. Journal code: 2985120R. ISSN: 0021-9193. Pub. country: United States. Language: English.

AB The toxR gene of Vibrio cholerae encodes a transcriptional activator required for the expression of the cholera toxin genes (ctxAB) and more than 15 other genes encoding secreted or membrane proteins. The latter group includes virulence genes involved in the biogenesis of the TCP pilus, the accessory colonization factor, and such ToxR-activated genes as tagA, mutations in which cause no detectable virulence defect in the suckling mouse model. To analyze the regulation of expression and the structure of tagA, we have cloned and sequenced about 2 kb of DNA upstream from a tagA::TnphoA fusion. While the portion of the tagA gene product examined presented no extensive similarity to any known protein, the amino acid sequence deduced from an open reading frame (designated aldA) located upstream from and in opposite orientation to tagA was highly similar to the sequences of eukaryotic aldehyde dehydrogenases. An assay of aldehyde dehydrogenase activity in extracts of a wild-type V. cholerae strainand an

aldA mutant confirmed that aldA encodes an aldehyde dehydrogenase. Expression of the aldA gene was studied together with that of tagA in both V. cholerae and Escherichia coli. The expression of both tagA and aldA was environmentally regulated and dependent on a functional toxR gene in V. cholerae, but neither promoter was activated by ToxR in E. coli, suggesting that expression of tagA and aldA requires an additional transcriptional activator besides ToxR. The aldA gene is the first example of a gene encoding a cytoplasmic protein that is under the control of ToxR, and this suggests that metabolic enzymes may constitute novel members of virulence regulons in bacteria.

L20 ANSWER 117 OF 128 MEDLINE DUPLICATE 57
92048495 Document Number: 92048495. PubMed ID: 1943708. Analysis of structure and function of the B subunit of cholera toxin by the use of site-directed mutagenesis. Jobling M G; Holmes R K. (Department of Microbiology, F. Edward Herbert School of Medicine, Uniformed Services University of the Health Sciences, Bethesda, Maryland 20814-4799.) MOLECULAR MICROBIOLOGY, (1991 Jul) 5 (7) 1755-67. Journal code: 8712028. ISSN: 0950-382X. Pub. country: ENGLAND: United Kingdom. Language: English.
AB Oligonucleotide-directed mutagenesis of **ctxB** was used to produce mutants of cholera toxin B subunit (CT-B) altered at residues Cys-9, Gly-33, Lys-34, Arg-35, Cys-86 and Trp-88. Mutants were identified phenotypically by radial passive immune haemolysis assays and genotypically by colony hybridization with specific oligonucleotide probes. Mutant CT-B polypeptides were characterized for immunoreactivity, binding to ganglioside GM1, ability to associate with the A subunit, ability to form holotoxin, and biological activity. Amino acid substitutions that caused decreased binding of mutant CT-B to ganglioside GM1 and abolished toxicity included negatively charged or large hydrophobic residues for Gly-33 and negatively or positively charged residues for Trp-88. Substitution of lysine or arginine for Gly-33 did not affect immunoreactivity or GM1-binding activity of CT-B but abolished or reduced toxicity of the mutant holotoxins, respectively. Substitutions of Glu or Asp for Arg-35 interfered with formation of holotoxin, but none of the observed substitutions for Lys-34 or Arg-35 affected binding of CT-B to GM1. The Cys-9, Cys-86 and Trp-88 residues were important for establishing or maintaining the native conformation of CT-B or protecting the CT-B polypeptide from rapid degradation in vivo.

L20 ANSWER 118 OF 128 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
1991:432791 Document No.: BA92:88956. ANALYSIS OF STRUCTURE AND FUNCTION OF THE B SUBUNIT OF CHOLERA TOXIN BY THE USE OF SITE-DIRECTED MUTAGENESIS. JOBLING M G; HOLMES R K. DEP. MICROBIOL., F. EDWARD HERBERT SCH. MED., UNIF. SERV. UNIV. HEALTH SCI., 4301 JONES BRIDGE ROAD, BETHESDA, MD. 20814-4799, USA.. MOL MICROBIOL, (1991) 55 (7), 1755-1768. CODEN: MOMIEE. ISSN: 0950-382X. Language: English.
AB Oligonucleotide-directed mutagenesis of **ctxB** was used to produce mutants of cholera toxin B subunit (CT-B) altered at residues Cys-9, Gly-33, Lys-34, Arg-35, Cys-86 and Trp-88. Mutants were identified phenotypically by radial passive immune haemolysis assays and genotypically by colony hybridization with specific oligonucleotide probes. Mutant CT-B polypeptides were characterized for immunoreactivity, binding to ganglioside GM1, ability to associate with the A subunit, ability to form holotoxin, and biological activity. Amino acid substitutions that caused decreased binding of mutant CT-B to ganglioside GM1 and abolished toxicity included negatively charged or large hydrophobic residues for Gly-33 and negatively or positively charged residues for Trp-88. Substitution of lysine or arginine for Gly-33 did not affect immunoreactivity or GM1-binding activity of CT-B but abolished or reduced toxicity of the Glu or Asp for Arg-35 interfered with formation of holotoxin, but none of the observed substitutions for Lys-34 or Arg-35 affected binding of Ct-B to M1. The Cys-9, Cys-86 and Trp-88 residues were important for establishing or maintaining the native conformation of CT-B

or protecting the CT-B polypeptide from rapid degradation in vivo.

L20 ANSWER 119 OF 128 CAPLUS COPYRIGHT 2002 ACS
1991:115998 Document No. 114:115998 Enterotoxin-associated DNA sequence homology between Salmonella species and Escherichia coli. Chopra, A. K.; Peterson, J. W.; Houston, C. W.; Pericas, R.; Prasad, R. (Dep. Microbiol., Univ. Texas, Galveston, TX, 77550, USA). FEMS Microbiol. Lett., 77(2-3), 133-8 (English) 1991. CODEN: FMLED7. ISSN: 0378-1097.
AB Multiple HindIII-restriction fragments of Salmonella typhimurium and S. typhi chromosomal DNA exhibited homol. with the heat-labile enterotoxin (LT1) gene of E. coli as detd. by Southern blot anal. A 9.4 kb HindIII restriction fragment identified in S. typhimurium and S. typhi chromosomal DNA reacted with both eltA and eltB gene probes. However, the homol. of the 9.4 kb DNA fragment from these Salmonella species was greater with eltB than eltA. In addn., a synthetic oligonucleotide probe, made to a portion of the putative GM1-ganglioside binding region of cholera toxin (CT) and LT1, hybridized with the 9.4 kb DNA fragment of S. typhimurium but not with the 9.4 kb fragment found in S. typhi isolates. The hybridization of multiple restriction fragments of Salmonella DNA with eltA and eltB gene sequences further suggests duplication of the stx operon on the chromosome of these bacteria.

L20 ANSWER 120 OF 128 MEDLINE DUPLICATE 58
90316661 Document Number: 90316661. PubMed ID: 2370100. Oral immunization of mice with a live recombinant Yersinia enterocolitica O:9 strain that produces the cholera toxin B subunit. Sory M P; Hermand P; Vaerman J P; Cornelis G R. (Unite de Microbiologie, Universite Catholique de Louvain, Belgium.) INFECTION AND IMMUNITY, (1990 Aug) 58 (8) 2420-8. Journal code: 0246127. ISSN: 0019-9567. Pub. country: United States. Language: English.
AB The 70-kilobase pYV plasmid of Yersinia enterocolitica encodes a set of proteins called Yops that are produced during infection. To use Y. enterocolitica as a live carrier to present the cholera toxin B (CT-B) subunit to the immune system, we constructed an operon fusion between **ctxB** and the yop51 gene. This operon fusion was either cloned on an RSF1010-derived plasmid or integrated into the pYV plasmid itself. In Y. enterocolitica, both constructions directed the synthesis of free CT-B only under conditions of Yops production, i.e., at 37 degrees C in a medium deprived of Ca2+. Bacteria containing both types of recombinant plasmids were given orally to mice. A serum antibody response against CT-B was detected in both cases. A secretory immunoglobulin A activity specific to CT-B was also observed in the intestinal secretions. According to immunoblot analysis, the serum antibody response was only directed against the polymeric form of the B subunit. The **ctxB** gene was also inserted in frame within yop51, giving a chimeric Yop51-CT-B protein that was secreted into the surrounding medium. In this case, however, no antibody response was observed after oral inoculation of mice. This lack of response probably results from the inability of the hybrid protein to assemble into the polymeric form of the B subunit.

L20 ANSWER 121 OF 128 MEDLINE DUPLICATE 59
90269236 Document Number: 90269236. PubMed ID: 2189728. Extracellular transport of cholera toxin B subunit using Neisseria IgA protease beta-domain: conformation-dependent outer membrane translocation. Klauser T; Pohlner J; Meyer T F. (Max-Planck-Institut fur Biologie, Abteilung Infektionsbiologie, Tubingen, FRG.) EMBO JOURNAL, (1990 Jun) 9 (6) 1991-9. Journal code: 8208664. ISSN: 0261-4189. Pub. country: ENGLAND: United Kingdom. Language: English.
AB The beta-domain of the Neisseria IgA protease precursor (Iga) provides the essential transport function for the protease across the outer membrane. To investigate the secretion function of the beta-domain (Iga beta), we engineered hybrid proteins between Iga beta and the non-toxic 12 kd cholera toxin B subunit (**CtxB**) and examined their targeting

behaviour in Salmonella typhimurium. We show that **CtxB**-Iga beta hybrid proteins integrate into the outer membrane, leading to the exposition of the **CtxB** moiety on the cell surface. Exposed **CtxB** can be degraded by externally added proteases like trypsin, but can also be specifically cleaved off from membrane-associated Iga beta by purified IgA protease. We further demonstrate that folding of the **CtxB** moiety at the periplasmic side of the outer membrane interferes with its translocation. Prevention of disulphide-induced folding in periplasmic **CtxB** renders the protein moiety competent for outer membrane transport. Iga beta may be of general interest as an export vehicle for even larger proteins from Gram-negative bacteria.

L20 ANSWER 122 OF 128 MEDLINE DUPLICATE 60
91326975 Document Number: 91326975. PubMed ID: 2101483. Delivery of the cholera toxin B subunit by using a recombinant Yersinia enterocolitica strain as a live oral carrier. Sory M P; Cornelis G R. (Unite de Microbiologie, Universite Catholique de Louvain, Brussels.) RESEARCH IN MICROBIOLOGY, (1990 Sep-Oct) 141 (7-8) 921-9. Journal code: 8907468. ISSN: 0923-2508. Pub. country: France. Language: English.

AB The gene **ctxB** encoding the cholera toxin B subunit was subcloned to design its production by Yersinia enterocolitica. It was joined in two ways to yopH, a gene of the virulence plasmid pYV specific to this genus. This gene encodes one of the major Yop proteins (YopH) secreted by bacteria incubated at 37 degrees C in a Ca(2+)-deprived medium. In a first construction, an operon fusion was obtained between **ctxB** and yopH so that CT-B and a truncated YopH protein were produced. The recombinant CT-B from Y. enterocolitica was structurally and antigenically similar to CT-B produced by Vibrio cholerae. In another construction, the fusion gene obtained directed the production of YopH'/CT-B hybrid proteins that were secreted by Y. enterocolitica. In both cases, Y. enterocolitica directed the production of the recombinant proteins only when the bacteria were incubated in conditions of Yops production. When bacteria carrying the operon fusion were given orally to mice, a clear serum antibody response against CT-B was detected by ELISA. According to immunoblot analysis, this response was only directed against the polymeric form of the B subunit.

L20 ANSWER 123 OF 128 MEDLINE DUPLICATE 61
90323604 Document Number: 90323604. PubMed ID: 2197181. Expression in Escherichia coli of two mutated genes encoding the cholera toxin B subunit. L'hoir C; Renard A; Martial J A. (Laboratoire Central de Genie Genetique, Universite de Liege, Belgium.) GENE, (1990 Apr 30) 89 (1) 47-52. Journal code: 7706761. ISSN: 0378-1119. Pub. country: Netherlands. Language: English.

AB To allow subsequent genetically mediated fusion of foreign antigens to cholera toxin B subunit (CTB), two mutated CTB encoding genes (**ctxB**) were constructed and overexpressed in Escherichia coli. The signal peptide coding sequence was deleted and restriction sites were created at both ends of the modified sequence. Both synthesized CTBs contain additional amino acid(s) at the N terminus (one and three). They were purified as insoluble products and refolded into the natural pentameric CTB structure by a denaturation-renaturation cycle. After renaturation, both recombinant proteins recovered CTB antigenicity and the ability to bind to GM1 gangliosides, as shown by in vitro analysis. Preliminary data indicated that both properties were unaltered by fusion of a foreign peptide to the mutated CTBs.

L20 ANSWER 124 OF 128 MEDLINE DUPLICATE 62
90060824 Document Number: 90060824. PubMed ID: 2531107. Plasmid vectors for constructing translational fusions to the B subunit of cholera toxin. Dertzbaugh M T; Macrina F L. (Department of Microbiology and Immunology, Virginia Commonwealth University, Richmond 23298-0678.) GENE, (1989 Oct 30) 82 (2) 335-42. Journal code: 7706761. ISSN: 0378-1119. Pub. country:

Netherlands. Language: English.

AB A family of plasmid cloning vectors has been developed for creating translational fusions to the **ctxB** gene encoding the B subunit of cholera toxin (CTB) in Escherichia coli. These vectors permit insertion of transcriptionally and translationally competent gene sequences upstream from **ctxB**. To test the utility of the system, a portion of the glucosyltransferase B (GTF) gene (gtfB) from the cariogenic bacterium Streptococcus mutans GS-5 (Bratthall serotype c), encoding the N-terminal one-third of the protein, was inserted into each vector. E. coli lysates containing the constructs were partially purified by passage over a GM1 ganglioside affinity column. Western blotting analysis of the column retentate from one of the lysates revealed the presence of a novel 58-kDa protein which cross-reacted with antisera to GTF and CTB. These vectors are of general use for making other translational fusions to **ctxB** . The high binding affinity of CTB can be exploited in purifying large polypeptides fused to this relatively small protein. Moreover, these vectors can be used to create neoantigens with altered immunogenicity for use in polypeptide-based vaccines.

L20 ANSWER 125 OF 128 MEDLINE DUPLICATE 63

88032830 Document Number: 88032830. PubMed ID: 2822664. Cloning and expression of the Salmonella enterotoxin gene. Chopra A K; Houston C W; Peterson J W; Prasad R; Mekalanos J J. (Department of Microbiology, University of Texas Medical Branch, Galveston 77550.) JOURNAL OF BACTERIOLOGY, (1987 Nov) 169 (11) 5095-100. Journal code: 2985120R. ISSN: 0021-9193. Pub. country: United States. Language: English.

AB This report examines the genetic basis for Salmonella typhimurium Q1 enterotoxin production. A 918-base-pair XbaI-HincII fragment of plasmid pJM17, composed of cholera toxin (CT) coding sequences (ctxAB), was used as a gene probe. With this probe, the S. typhimurium enterotoxin was identified on a 6.3-kilobase EcoRI-PstI fragment of chromosomal DNA from plasmidless strain Q1. We cloned this 6.3-kilobase fragment into Escherichia coli RR1. The genetic map of the cloned Salmonella enterotoxin (stx) gene was similar but not identical to the CT and E. coli heat-labile enterotoxin genes. By using synthetic oligonucleotides derived from the sequences of CT subunits A (ctxA) and B (**ctxB**), it was revealed that there were some conserved regions of DNA encoding the enterotoxins of strain Q1 and Vibrio cholerae. Expression of the cloned stx gene in minicells and subsequent Western blot (immunoblot) analysis with CT antitoxin demonstrated that the Salmonella enterotoxin had two or more subunits with molecular sizes of 45, 26, and 12 kilodaltons. Crude cell lysates of E. coli RR1(pCHP4), containing the cloned Salmonella enterotoxin gene, elicited fluid secretion in ligated rabbit intestinal loops and firm induration in rabbit skin. Both of these enterotoxic responses were neutralized by antisera specific for CT. Mucosal tissue from positive intestinal loops contained elevated levels of cyclic AMP. These data suggest some evolutionary relatedness between the enterotoxin genes of S. typhimurium and V. cholerae.

L20 ANSWER 126 OF 128 CAPLUS COPYRIGHT 2002 ACS

1985:57165 Document No. 102:57165 Plasmids and modified strains of Vibrio cholerae and derived vaccines. Mekalanos, John J. (Harvard College, USA). Eur. Pat. Appl. EP 125228 A1 19841114, 23 pp. DESIGNATED STATES: R: AT, BE, CH, DE, FR, GB, IT, LI, LU, NL, SE. (English). CODEN: EPXXDW. APPLICATION: EP 1984-870058 19840426. PRIORITY: US 1983-489958 19830429.

AB A V. cholerae strain that contains a deletion in both copies of the A1-peptide gene (ctxA), and thus, is unable to express the toxic A1 peptide, is constructed by mol. genetic methods. The mutant strain, which is capable of colonizing the intestine, is immunogenic, nontoxinogenic, and may be used as either a live or dead cholera vaccine. Thus, plasmid pJM17, which carries the ctxA gene (for toxin) and the **ctxB** gene (required for cell membrane attachment and transport), as well as a selectable tetracycline-resistance marker, was isolated from wild-type V.

cholerae, and the linearized plasmid was treated with XbaI and Bal31. The mutant plasmid, pJM23, had a deletion of the ctxA sequence that encoded amino acids 10-164, i.e. >80%, of the protein. Plasmid pJM23, or 1 of its derivs., was recombined with V. cholerae strain Ogawa 0395, which is immunogenic, and recombination of the deleted sequence with the 0395 chromosome was selected. The deletion mutation was spontaneously transferred to the 2nd copy of ctxA in the 0395 genome by growing 0395 for several generations. The 0395 derivs. that carried the deletion mutation in both copies of ctxA were identified by colony hybridization with a XbaI/HincII fragment of pJM17that specifically hybridizes to the DNA which is absent from the deletion mutation. After the plasmid was cured, strain 0395-N1 (contg. the double gene ctxA deletion, the **ctxB** sequence, and no plasmid) was suitable for use as a vaccine against cholera.

L20 ANSWER 127 OF 128 CAPLUS COPYRIGHT 2002 ACS

1984:484904 Document No. 101:84904 Synthesis of cholera toxin is positively regulated at the transcriptional level by toxR. Miller, Virginia L.; Mekalanos, John J. (Dep. Microbiol. Mol. Genet., Harvard Med. Sch., Boston, MA, 02115, USA). Proc. Natl. Acad. Sci. U. S. A., 81(11), 3471-5 (English) 1984. CODEN: PNASA6. ISSN: 0027-8424.

AB A pos. regulatory gene (toxR) from Vibrio cholerae was cloned that controls cholera toxin transcription. This was done by 1st constructing a genetic fusion consisting of the lacZ gene fused to the promoter of the cholera toxin operon ctxAB. This operon fusion was used to screen a V. cholerae genomic library for genes that could activate the ctx promoter in Escherichia coli. This method allowed the identification of a gene, toxR, that increases ctx expression by >100-fold. Complementation anal. indicated that certain hypotoxinogenic mutants of V. cholerae 569B probably have mutations in the toxR gene. Southern blot anal. suggests that all V. cholerae, including nontoxinogenic strains, have the toxR gene. Moreover, nontoxinogenic strains not only lack the structural genes for cholera toxin but also sequences assocd. with the larger 7-kilobase ctx genetic element.

L20 ANSWER 128 OF 128 CAPLUS COPYRIGHT 2002 ACS

1984:144966 Document No. 100:144966 Preparation of DNA sequences and recombinant DNA molecules coding for the cholera toxin subunits A and B and compositions containing the subunit(s) obtained. Harford, Nigel; De Wilde, Michel (Smith Kline-Rit S. A., Belg.). Eur. Pat. Appl. EP 95452 A2 19831130, 46 pp. DESIGNATED STATES: R: AT, BE, CH, DE, FR, GB, IT, LI, LU, NL, SE. (French). CODEN: EPXXDW. APPLICATION: EP 1983-870048 19830518. PRIORITY: US 1982-381083 19820524.

AB DNA sequences and recombinant DNA mols. contg. a region coding for all or part of subunits A and (or) B of cholera toxin are prepd. from DNA of Vibrio cholerae. Cholera toxin subunits are formed in microorganisms transformed with recombinant plasmids. The prepn. of a vaccine effective against V. cholerae is described. Thus, DNA was isolated from V. cholerae eltor INABA (ATCC 39050) and cleared with ClaI. DNA fragments encoding the A and B subunits of cholera toxin (ctxA and **ctxB**, resp.) were identified by Southern hybridizations with genes eltA and eltB, which encode the thermolabile enterotoxin of Escherichia coli. Genes ctxA and **ctxB** were cloned in plasmid pBR322 in E. coli to yield the recombinant plasmids pRIT10841 and pRIT10810, resp., in the E. coli strains ATCC 39053 and ATCC 39051, resp. Plasmid pRIT10814, which contained ctxA and **ctxB** was constructed by the insertion of a ClaI-BglII fragment of pRIT10810 into plasmid pBR327 cleared with ClaI and BamHI to yield pRIT10812. The last plasmid was cleared with ClaI and a ClaI fragment of pRIT10841 was inserted to yield pRIT0814, which was present in E. coli strain ATCC 39052. Exts. of ATCC 39052 gave pos. ileal-loop tests (indicating liq. accumulation) in 9 of 10 rabbits injected. Purified cholera toxin (100 ng) gave a pos. response in 8 of 8 rabbits tested; 10 ng of purified toxin gave a pos. response in 3 of 8

rabbits injected. Thus, genes ctxA and **ctxB**, which were joined by their common ClaI site on pRIT10814, yielded a functional determinant for cholera holotoxin. A PstI fragment that contained the 2 genes ctxA and **ctxB** was cloned (in pBR322) directly from DNA of V. cholerae ATCC 39050 to yield the plasmid pRIT10824. No difference in restriction sites in and around the 2 ctx genes was obsd. between plasmids pRIT10824 and pRIT10841. The sequences of ctxA and **ctxB** genes were detd., and the prepn. of an oral vaccine with cholera toxin subunit B produced in E. coli ATCC 39051 was described.

```
=> s "EtxB"
L22         193 "ETXB"

=> s l22 and allergy
L23           1 L22 AND ALLERGY

=> d l23 cbib abs

L23  ANSWER 1 OF 1  CAPLUS  COPYRIGHT 2002 ACS
1999:451202   Document No. 131:82960  EtxB or ganglioside GM1 for
     treating allergic or hypersensitivity conditions. Williams, Neil Andrew;
     Hirst, Timothy Raymond; Bienenstock, John (Oratol Limited, UK).  PCT Int.
     Appl. WO 9934817 A1 19990715, 46 pp.  DESIGNATED STATES: W:  AL, AM, AT,
     AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB,
     GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK,
     LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD,
     SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, AM, AZ,
     BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY,
     DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE,
     SN, TD, TG.  (English).  CODEN: PIXXD2.  APPLICATION: WO 1999-GB70
     19990108.  PRIORITY: GB 1998-487 19980109.
AB   The use of an agent in the manuf. of a medicament to treat an allergic
     condition and/or a hypersensitivity condition is described.  The agent is
     capable of modulating a ganglioside-assocd. activity.  The agent is not
     coupled to an antigen.  The modulation of the ganglioside-assocd. activity
     affects an allergic condition and/or a hypersensitivity condition.
     Examples of such modulators include ganglioside GM1 and E. coli
     enterotoxin B subunit.


=> s l22 and mutant
L24          93 L22 AND MUTANT

=> dup remove
ENTER L# LIST OR (END):124
PROCESSING COMPLETED FOR L24
L25           24 DUP REMOVE L24 (69 DUPLICATES REMOVED)

=> d l25 1-24 cbib abs

L25  ANSWER 1 OF 24  EMBASE  COPYRIGHT 2002 ELSEVIER SCI. B.V.
2002207943  EMBASE  Escherichia coli heat-labile enterotoxin B subunit prevents
     autoimmune arthritis through induction of regulatory CD4+ T cells. Luross
     J.A.; Heaton T.; Hirst T.R.; Day M.J.; Williams N.A.. Dr. N.A. Williams,
     University of Bristol, Department of Pathology, School of Medical
     Sciences, University Walk, Bristol BS8 1TD, United Kingdom.
     neil.a.williams@bristol.ac.uk. Arthritis and Rheumatism  46/6 (1671-1682)
     2002.
     Refs: 41.
     ISSN: 0004-3591. CODEN: ARHEAW. Pub. Country: United States. Language:
     English. Summary Language: English.
AB   Objective. The receptor-binding B subunit of Escherichia coli heat-labile
```

enterotoxin (**EtxB**) is a highly stable, nontoxic protein that is capable of modulating immune responses. This study was conducted to determine whether mucosal administration of **EtxB** can block collagen-induced arthritis (CIA) and to investigate the mechanisms involved. Methods. Clinical arthritis in DBA/1 mice was monitored following mucosal administration of **EtxB** on 4 occasions. The dependence of disease prevention on receptor binding by **EtxB** and the associated alterations to the immune response to type II collagen (CII) were assessed. Adoptive transfer experiments and lymph node cell cocultures were used to investigate the underlying mechanisms. Results. Both intranasal and intragastric delivery of **EtxB** were effective in preventing CIA; a 1-.mu.g dose of **EtxB** was protective after intranasal administration. A non-receptor-binding **mutant** of **EtxB** failed to prevent disease. Intranasal **EtxB** lowered both the incidence and severity of arthritis when given either at the time of disease induction or 25 days later. **EtxB** markedly reduced levels of anti-CII IgG2a antibodies and interferon-.gamma. (IFN.gamma.) production while not affecting levels of IgG1, interleukin-4 (IL-4), or IL-10. Disease protection could be transferred by CD4+ T cells from treated mice, an effect that was abrogated upon depletion of the CD25+ population. In addition, CD4+CD25+ T cells from treated mice were able to suppress anti-CII IFN.gamma. production by CII-primed lymph node cells. Conclusion. Mucosal administration of **EtxB** can be used to prevent or treat CIA. Modulation of the anti-CII immune response by **EtxB** is associated with a reduction in Th1 cell reactivity without a concomitant shift toward Th2. Instead, **EtxB** mediates its effects through enhancing the activity of a population of CD4+ regulatory T cells.

L25 ANSWER 2 OF 24 MEDLINE DUPLICATE 1

2002150343 Document Number: 21877323. PubMed ID: 11882700. Contribution of the ADP-ribosylating and receptor-binding properties of cholera-like enterotoxins in modulating cytokine secretion by human intestinal epithelial cells. Soriani Marco; Bailey Lorna; Hirst Timothy R. (Department of Pathology and Microbiology, University of Bristol, Bristol BS8 1TD, UK.) MICROBIOLOGY, (2002 Mar) 148 (Pt 3) 667-76. Journal code: 9430468. ISSN: 1350-0872. Pub. country: England: United Kingdom. Language: English.

AB When epithelial cells first encounter cholera toxin (Ctx) produced by Vibrio cholerae they secrete not only chloride ions responsible for causing diarrhoea, but also a number of cytokines that may contribute to the toxin's potent immunomodulatory properties. Much less is known about the ability of the heat-labile enterotoxin of Escherichia coli (Etx), a close homologue of Ctx, to elicit cytokine secretion by epithelial cells. This study shows that treatment of human intestinal epithelial T84 cells with Etx induces expression of IL-6, IL-10, IL-1R antagonist, as well as IL-1alpha and IL-1beta and low levels of IL-8. Such induction was totally dependent on the intrinsic ADP-ribosylating activity of the toxin A-subunit, and could be mimicked by cAMP-elevating agents, such as forskolin and dibutyryl cAMP. By comparison, neither an enzymically inactive **mutant** of Etx nor **EtxB** was able to induce cytokine secretion. The behaviour of Ctx and CtxB was very similar to that of Etx and **EtxB**, respectively. The spectrum of cytokines released by Etx and Ctx indicates that the toxins may create a local microenvironment that strongly biases the immune response towards an anti-inflammatory and a polarized Th2 response.

L25 ANSWER 3 OF 24 MEDLINE DUPLICATE 2

2002298918 Document Number: 22035445. PubMed ID: 12039916. Modulation of B lymphocyte signalling by the B subunit of Escherichia coli heat-labile enterotoxin. Bone Heather; Eckholdt Stephanie; Williams Neil A. (Department of Pathology and Microbiology, School of Medical Sciences, University of Bristol, University Walk, Bristol BS8 1TD, UK.)

INTERNATIONAL IMMUNOLOGY, (2002 Jun) 14 (6) 647-58. Journal code: 8916182. ISSN: 0953-8178. Pub. country: England: United Kingdom. Language: English.

AB The non-toxic B subunit of Escherichia coli heat-labile enterotoxin (**EtxB**) is a potent mucosal adjuvant and immunomodulator capable of blocking autoimmune disease. These effects are linked with its ability to modulate lymphocyte populations-a feature that is dependent on binding to ubiquitously expressed cell surface receptors. Here, we demonstrate that **EtxB** can trigger up-regulated expression of class II MHC and CD25 on purified populations of B lymphocytes, suggesting that **EtxB** can directly activate biochemical signalling pathways in these cells. The nature of the intracellular signalling events was investigated. B cells cultured with **EtxB**, but not a non-receptor binding **mutant** protein, **EtxB**(G33D), caused the activation of the extracellular signal-regulated kinase (Erk) forms of mitogen-activated protein (MAP) kinase in a process that was dependent on MAPK/Erk kinase (MEK), phosphoinositide 3-kinase (PI3-kinase) and protein kinase C (PKC), as determined by the use of specific inhibitors. PI3-kinase was critical not only in the activation of MAP kinase but also in the up-regulation of both class II and CD25. However, MEK inhibition only partially abrogated the **EtxB**-mediated up-regulation of MHC class II expression and did not affect CD25 expression-findings suggesting that additional pathways downstream of PI3-kinase are involved. A role for PKC in these processes was suggested by the finding that inhibitors of PKC completely blocked **EtxB**-mediated CD25 up-regulation. Thus, we have shown that receptor binding by **EtxB** triggers multiple signalling pathways in B cells that regulate the expression of key cell surface molecules.

L25 ANSWER 4 OF 24 MEDLINE DUPLICATE 3

2001419634 Document Number: 21353030. PubMed ID: 11447291. A **mutant** cholera toxin B subunit that binds GM1- ganglioside but lacks immunomodulatory or toxic activity. Aman A T; Fraser S; Merritt E A; Rodigherio C; Kenny M; Ahn M; Hol W G; Williams N A; Lencer W I; Hirst T R. (Department of Pathology and Microbiology, University of Bristol, Bristol BS81TD, United Kingdom.) PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (2001 Jul 17) 98 (15) 8536-41. Journal code: 7505876. ISSN: 0027-8424. Pub. country: United States. Language: English.

AB GM1-ganglioside receptor binding by the B subunit of cholera toxin (CtxB) is widely accepted to initiate toxin action by triggering uptake and delivery of the toxin A subunit into cells. More recently, GM1 binding by isolated CtxB, or the related B subunit of Escherichia coli heat-labile enterotoxin (**EtxB**), has been found to modulate leukocyte function, resulting in the down-regulation of proinflammatory immune responses that cause autoimmune disorders such as rheumatoid arthritis and diabetes. Here, we demonstrate that GM1 binding, contrary to expectation, is not sufficient to initiate toxin action. We report the engineering and crystallographic structure of a **mutant** cholera toxin, with a His to Ala substitution in the B subunit at position 57. Whereas the **mutant** retained pentameric stability and high affinity binding to GM1-ganglioside, it had lost its immunomodulatory activity and, when part of the holotoxin complex, exhibited ablated toxicity. The implications of these findings on the mode of action of cholera toxin are discussed.

L25 ANSWER 5 OF 24 MEDLINE DUPLICATE 4

2001392800 Document Number: 21340378. PubMed ID: 11447169. Escherichia coli enterotoxin B subunit triggers apoptosis of CD8(+) T cells by activating transcription factor c-myc. Soriani M; Williams N A; Hirst T R. (Department of Pathology and Microbiology, University of Bristol, Bristol, BS8 1TD, United Kingdom.) INFECTION AND IMMUNITY, (2001 Aug) 69 (8) 4923-30. Journal code: 0246127. ISSN: 0019-9567. Pub. country: United States. Language: English.

AB Heat-labile enterotoxin from enterotoxinogenic Escherichia coli is not only an important cause of diarrhea in humans and domestic animals but also possesses potent immunomodulatory properties. Recently, the nontoxic, receptor-binding B subunit of heat-labile enterotoxin (**EtxB**) was found to induce the selective death of CD8(+) T cells, suggesting that **EtxB** may trigger activation of proapoptotic signaling pathways. Here we show that **EtxB** treatment of CD8(+) T cells but not of CD4(+) T cells triggers the specific up-regulation of the transcription factor c-myc, implicated in the control of cell proliferation, differentiation, and death. A concomitant elevation in Myc protein levels was also evident, with peak expression occurring 4 h posttreatment. Preincubation with c-myc antisense oligodeoxynucleotides demonstrated that Myc expression was necessary for **EtxB**-mediated apoptosis. Myc activation was also associated with an increase of IkappaBalpha turnover, suggesting that elevated Myc expression may be dependent on NF-kappaB. When CD8(+) T cells were pretreated with inhibitors of IkappaBalpha turnover and NF-kappaB translocation, this resulted in a marked reduction in both **EtxB**-induced apoptosis and Myc expression. Further, a non-receptor-binding **mutant** of **EtxB, EtxB** (G33D), was shown to lack the capacity to activate Myc transcription. These findings provide further evidence that **EtxB** is a signaling molecule that triggers activation of transcription factors involved in cell survival.

L25 ANSWER 6 OF 24 SCISEARCH COPYRIGHT 2002 ISI (R)
2001:338101 The Genuine Article (R) Number: 423CT. Escherichia coli heat-labile enterotoxin B subunit is a more potent mucosal adjuvant than its closely related homologue, the B subunit of cholera toxin. Millar D G (Reprint); Hirst T R; Snider D P. Ontario Canc Inst, Dept Med Biophys, 610 Univ Ave, Room 8-318, Toronto, ON M5G 2M9, Canada (Reprint); McMaster Univ, Dept Pathol & Mol Med, Hamilton, ON L8N 3Z5, Canada; Univ Bristol, Dept Pathol & Microbiol, Bristol, Avon, England. INFECTION AND IMMUNITY (MAY 2001) Vol. 69, No. 5, pp. 3476-3482. Publisher: AMER SOC MICROBIOLOGY . 1752 N ST NW, WASHINGTON, DC 20036-2904 USA. ISSN: 0019-9567. Pub. country: Canada; England. Language: English.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS
AB Although cholera toxin (Ch) and Escherichia coli heat-labile enterotoxin (Etx) are known to be potent mucosal adjuvants, it remains controversial whether the adjuvanticity of the holotoxins extends to their nontoxic, receptor-binding B subunits, Here, ave halle systematically evaluated the comparative adjuvant properties of highly purified recombinant **EtxB** and CtxB, **EtxB** was found to be a more potent adjuvant than CtxB, stimulating responses to hell egg lysozyme when the two were coadministered to mice intranasally, as assessed by enhanced serum and secretory antibody titers as well as by stimulation of lymphocyte proliferation in spleen and draining lymph nodes. These results indicate that, although structurally very similar, **EtxB** and CtxB have strikingly different immunostimulatory propel ties and should not be considered equivalent as prospective vaccine adjuvants.

L25 ANSWER 7 OF 24 MEDLINE DUPLICATE 5
2001354948 Document Number: 21178697. PubMed ID: 11282993. Evidence for a role of ganglioside GM1 in antigen presentation: binding enhances presentation of Escherichia coli enterotoxin B subunit (**EtxB**) to CD4(+) T cells. Nashar T O; Betteridge Z E; Mitchell R N. (Department of Pathology and Microbiology, School of Medical Sciences, University of Bristol, Bristol BS8 1TD, UK.) INTERNATIONAL IMMUNOLOGY, (2001 Apr) 13 (4) 541-51. Journal code: 8916182. ISSN: 0953-8178. Pub. country: England: United Kingdom. Language: English.
AB Successful antigen presentation by antigen-presenting cells is governed by a number of factors including the efficiency of antigen capture by cell-surface receptors, targeting to compartments of antigen processing, surface expression of MHC II-peptide complexes and presence of

co-stimulatory signals. Ganglioside GM1 is an important component of membrane glycosphingolipids, and has been implicated in cell differentiation, apoptosis and signal transduction pathways. Using the B subunit of Escherichia coli enterotoxin (**EtxB**), a potent immunogen that binds GM1 with high affinity, and a non-binding **mutant** of **EtxB**, **EtxB**(G33D), we demonstrate that GM1 is intimately involved in several aspects of antigen presentation. Thus, GM1-mediated presentation of **EtxB** by B cells and CD11c(+) dendritic cells (DC) significantly enhanced the proliferation and cytokine expression of **EtxB**-specific CD4(+) T cells. Investigation regarding potential mechanisms revealed that **EtxB** binding directly augments the expression of MHC class II on B cells, and fractionation of B cells demonstrated that **EtxB** binding to GM1 results in rapid internalization and targeting to class II-rich compartments. GM1-mediated uptake of antigens and access to class II compartments in B cells can be exploited to significantly enhance the presentation of ovalbumin-conjugated to **EtxB**. These results demonstrate that GM1 can play an important role in antigen presentation via the MHC II pathway.

L25 ANSWER 8 OF 24 MEDLINE DUPLICATE 6

1999134317 Document Number: 99134317. PubMed ID: 9933586. Structural basis for the differential toxicity of cholera toxin and Escherichia coli heat-labile enterotoxin. Construction of hybrid toxins identifies the A2-domain as the determinant of differential toxicity. Rodighiero C; Aman A T; Kenny M J; Moss J; Lencer W I; Hirst T R. (Department of Pathology and Microbiology, University of Bristol, School of Medical Sciences, Bristol BS8 1TD, United Kingdom.) JOURNAL OF BIOLOGICAL CHEMISTRY, (1999 Feb 12) 274 (7) 3962-9. Journal code: 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.

AB Cholera toxin (Ctx) and E. coli heat-labile enterotoxin (Etx) are structurally and functionally similar AB5 toxins with over 80% sequence identity. When their action in polarized human epithelial (T84) cells was monitored by measuring toxin-induced Cl- ion secretion, Ctx was found to be the more potent of the two toxins. Here, we examine the structural basis for this difference in toxicity by engineering a set of **mutant** and hybrid toxins and testing their activity in T84 cells. This revealed that the differential toxicity of Ctx and Etx was (i) not due to differences in the A-subunit's C-terminal KDEL targeting motif (which is RDEL in Etx), as a KDEL to RDEL substitution had no effect on cholera toxin activity; (ii) not attributable to the enzymatically active A1-fragment, as hybrid toxins in which the A1-fragment in Ctx was substituted for that of Etx (and vice versa) did not alter relative toxicity; and (iii) not due to the B-subunit, as the replacement of the B-subunit in Ctx for that of Etx caused no alteration in toxicity, thus excluding the possibility that the broader receptor specificity of **EtxB** is responsible for reduced activity. Remarkably, the difference in toxicity could be mapped to a 10-amino acid segment of the A2-fragment that penetrates the central pore of the B-subunit pentamer. A comparison of the in vitro stability of two hybrid toxins, differing only in this 10-amino acid segment, revealed that the Ctx A2-segment conferred a greater stability to the interaction between the A- and B-subunits than the corresponding segment from Etx A2. This suggests that the reason for the relative potency of Ctx compared with Etx stems from the increased ability of the A2-fragment of Ctx to maintain holotoxin stability during uptake and transport into intestinal epithelia.

L25 ANSWER 9 OF 24 MEDLINE DUPLICATE 7

97289759 Document Number: 97289759. PubMed ID: 9144230. Prevention of autoimmune disease due to lymphocyte modulation by the B-subunit of Escherichia coli heat-labile enterotoxin. Williams N A; Stasiuk L M; Nashar T O; Richards C M; Lang A K; Day M J; Hirst T R. (Department of Pathology and Microbiology, School of Medical Sciences, University of

Bristol, Bristol BS8 1TD, United Kingdom.) PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (1997 May 13) 94 (10) 5290-5. Journal code: 7505876. ISSN: 0027-8424. Pub. country: United States. Language: English.

AB We demonstrate that the receptor binding moiety of Escherichia coli heat-labile enterotoxin (**EtxB**) can completely prevent autoimmune disease in a murine model of arthritis. Injection of male DBA/1 mice at the base of the tail with type II collagen in the presence of complete Freund's adjuvant normally leads to arthritis, as evidenced by inflammatory infiltration and swelling of the joints. A separate injection of **EtxB** at the same time as collagen challenge prevented leukocyte infiltration, synovial hyperplasia, and degeneration of the articular cartilage and reduced clinical symptoms of disease by 82%. The principle biological property of **EtxB** is its ability to bind to the ubiquitous cell surface receptor GM1 ganglioside, and to other galactose-containing glycolipids and galactoproteins. The importance of receptor interaction in mediating protection from arthritis was demonstrated by the failure of a non-receptor-binding **mutant** of **EtxB** to elicit any protective effect. Analysis of T cell responses to collagen, in cultures of draining lymph node cells, revealed that protection was associated with a marked increase in interleukin 4 production concomitant with a reduction in interferon gamma levels. Furthermore, in protected mice there was a significant reduction in anti-collagen antibody levels as well as an increase in the IgG1/IgG2a ratio. These observations show that protection is associated with a shift in the Th1/Th2 balance as well as a general reduction in the extent of the anti-type II collagen immune response. This suggests that **EtxB** -receptor-mediated modulation of lymphocyte responses provides a means of preventing autoimmune disease.

L25 ANSWER 10 OF 24 MEDLINE DUPLICATE 8

1998018503 Document Number: 98018503. PubMed ID: 9378497. Modulation of B-cell activation by the B subunit of Escherichia coli enterotoxin: receptor interaction up-regulates MHC class II, B7, CD40, CD25 and ICAM-1. Nashar T O; Hirst T R; Williams N A. (School of Medical Sciences, University of Bristol, UK.) IMMUNOLOGY, (1997 Aug) 91 (4) 572-8. Journal code: 0374672. ISSN: 0019-2805. Pub. country: ENGLAND: United Kingdom. Language: English.

AB The B subunits of cholera toxin (CtxB) and Escherichia coli heat-labile enterotoxin (**EtxB**) are non-toxic lectins that bind and cross-link a ubiquitous cell glycolipid receptor, ganglioside GM1, and are recognized as potent mucosal and systemic immunogens. Here we examine the role of **EtxB** receptor occupancy in modulating the activation of B cells, in vitro, in primary lymphocyte cultures containing B and T cells. When 48-hr spleen cell cultures containing **EtxB** were compared with those in the presence of a non-receptor binding **mutant, EtxB**(G33D), a marked shift in the ratio of CD4+ T cells: B cells was noted. Evidence suggested that this was the result of either enhanced survival or proliferation of B cells associated with receptor occupancy by **EtxB**. Investigation revealed that **EtxB** induced only a minimal increase in proliferation above that of **EtxB**(G33D), in mixed cell cultures, and failed to induce any cell division of purified B cells or T cells. In contrast, receptor-binding by **EtxB** markedly up-regulated the expression of major histocompatability complex (MHC) class II, B7, intracellular adhesion molecule-1 (ICAM-1), CD40 and CD25 on the B-cell surface. These results indicate that the polyclonal effects of **EtxB** on B cells are not associated with wide-scale proliferation, but more likely with maintenance of B-cell survival by activation of molecules essential for B-cell differentiation. The findings also highlight the essential role of GM1-interaction with **EtxB** in the regulation of lymphocyte responses.

L25 ANSWER 11 OF 24 MEDLINE DUPLICATE 9

97128619 Document Number: 97128619. PubMed ID: 8973177. A pH-dependent conformational change in the B-subunit pentamer of Escherichia coli heat-labile enterotoxin: structural basis and possible functional role for a conserved feature of the AB5 toxin family. Ruddock L W; Webb H M; Ruston S P; Cheesman C; Freedman R B; Hirst T R. (Research School of Biosciences, University of Kent at Canterbury, U.K.. l.w.ruddock@ukc.ac.uk) . BIOCHEMISTRY, (1996 Dec 17) 35 (50) 16069-76. Journal code: 0370623. ISSN: 0006-2960. Pub. country: United States. Language: English.

AB The non-covalently associated B-subunit moieties of AB5 toxins, such as cholera toxin and related diarrheagenic enterotoxins, exhibit exceptional pH stability and remain pentameric at pH values as low as 2.0. Here, we investigate the structural basis of a pH-dependent conformational change which occurs within the B5 structure of Escherichia coli heat-labile enterotoxin (**EtxB**) at around pH 5.0. The use of far-UV CD and fluorescence spectroscopy showed that **EtxB** pentamers undergo a fully reversible pH-dependent conformational change with a pKa of 4.9 +/- 0.1 (R2 = 0.999) or 5.13 +/- 0.01 (R2 = 0.999), respectively. This renders the pentamer susceptible to SDS-mediated disassembly and decreases its thermal stability by 18 degrees C. A comparison of the pH-dependence of the structural change in EtxB5, with that of a **mutant** containing a Ser substitution at His 57, revealed that the pKa of the conformational change was shifted from ca. 5.1 to 4.4. This finding suggests that protonation of the imidazole side chain of His 57 might facilitate disruption of a spatially adjacent salt bridge, located between Glu 51 and Lys 91 in each B-subunit, thus triggering the conformational change in the pentameric structure. The pH-dependent conformational change was found to be inhibited when B-subunits bound to monosialoganglioside, GMI; and to have no effect on the stability of interaction between A- and B-subunits within the AB5 complex. This suggests that the conformational change is unlikely to have a direct involvement in toxicity. Conservation of the pH-dependent conformational change in the AB5 toxin family, combined with the potential exposure of the hydrophobic core of beta-barrel in the monomeric units, leads to the proposal that the conformational change may be the common feature that ensures the secretion of these proteins from the Vibrionaceae.

L25 ANSWER 12 OF 24 MEDLINE DUPLICATE 10

96324796 Document Number: 96324796. PubMed ID: 8671661. Cross-linking of cell surface ganglioside GM1 induces the selective apoptosis of mature CD8+ T lymphocytes. Nahar T O; Williams N A; Hirst T R. (Research School of Biosciences, University of Kent, Canterbury, UK.) INTERNATIONAL IMMUNOLOGY, (1996 May) 8 (5) 731-6. Ref: 24. Journal code: 8916182. ISSN: 0953-8178. Pub. country: ENGLAND: United Kingdom. Language: English.

AB Gangliosides are glycosphingolipids found ubiquitously on the surface of mammalian cells. They contain a ceramide tail that is inserted into the membrane and exposed carbohydrate and sialic acid moieties. The non-toxic B subunit oligomer (**EtxB**) of Escherichia coli heat-labile enterotoxin (Etx) is a potent immunogen in vivo and has profound modulatory effects on **EtxB**-primed lymphocytes in vitro, properties which are dependent on its ability to bind to GM1 ganglioside receptors. Here, it is shown that cross-linking GM1 by **EtxB** causes a differential effect on mature CD4(+) and CD8(+) T cells from lymph node cultures proliferating in response to an unrelated antigen, ovalbumin. Addition of **EtxB** to such cultures led to the complete depletion of CD8(+) T cells compared with enhanced activation of CD4(+) cells [as measured by expression of CD25 (IL-2Ralpha)]. By contrast, addition of a **mutant EtxB**, **EtxB**(G33D), which does not bind to GM1, failed to trigger CD8(+) T cell depletion. When **EtxB** was added to isolated non-immune CD8(+) lymphocytes rapid (12-18 h) alterations in nuclear morphology and the appearance of sub-G0/G1 levels of DNA were induced; properties which are characteristic of cells undergoing apoptosis. **EtxB**(G33D) failed to trigger

apoptosis, indicating that the induction of the apoptotic signal was dependent on the binding of GM1. These findings provide an insight into the potent immunogenicity and immunomodulatory properties of E. coli enterotoxins as well as heralding a novel method for the selective induction of apoptosis in mature CD8(+) T lymphocytes.

L25 ANSWER 13 OF 24 MEDLINE DUPLICATE 11
96133910 Document Number: 96133910. PubMed ID: 8552610. Potent immunogenicity of the B subunits of Escherichia coli heat-labile enterotoxin: receptor binding is essential and induces differential modulation of lymphocyte subsets. Nashar T O; Webb H M; Eaglestone S; Williams N A; Hirst T R. (Research School of Biosciences, University of Kent, Canterbury, Great Britain.) PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (1996 Jan 9) 93 (1) 226-30. Journal code: 7505876. ISSN: 0027-8424. Pub. country: United States. Language: English.

AB The importance of receptor binding in the potent immunogenicity of Escherichia coli heat-labile enterotoxin B subunit (**EtxB**) was tested by comparing its immunogical properties with those of a receptor binding **mutant, EtxB**(G33D). Subcutaneous immunization of **EtxB**(G33D) resulted in 160-fold reduction in antibody titer compared with wild-type **EtxB**, whereas its oral delivery failed to provoke any detectable secretory or serum anti-B subunit responses. Moreover, the two proteins induced strikingly different effects on lymphocyte cultures in vitro. **EtxB**, in comparison with **EtxB**(G33D), caused an increase in the proportion of B cells, many of which were activated (CD25+); the complete depletion of CD8+ T cells; an increase in the activation of CD4+ T cells; and an increase in interleukin 2 and a decrease in interferon gamma. These data indicate that **EtxB** exerts profound effects on immune cells, suggesting that its potent immunogenicity is dependent not only on efficient receptor-mediated uptake, but also on direct receptor-mediated immunomodulation of lymphocyte subsets.

L25 ANSWER 14 OF 24 MEDLINE DUPLICATE 12
96102052 Document Number: 96102052. PubMed ID: 8530395. Kinetics of acid-mediated disassembly of the B subunit pentamer of Escherichia coli heat-labile enterotoxin. Molecular basis of pH stability. Ruddock L W; Ruston S P; Kelly S M; Price N C; Freedman R B; Hirst T R. (Biological Laboratory, University of Kent, Canterbury, United Kingdom.) JOURNAL OF BIOLOGICAL CHEMISTRY, (1995 Dec 15) 270 (50) 29953-8. Journal code: 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.

AB The B-subunit pentamer of Escherichia coli heat-labile enterotoxin (**EtxB**) is highly stable, maintaining its quaternary structure in a range of conditions that would normally be expected to cause protein denaturation. In this paper the structural stability of **EtxB** has been studied as a function of pH by electrophoretic, immunochemical, and spectroscopic techniques. Disassembly of the cyclic pentameric structure of human **EtxB** occurs only below pH 2. As determined by changes in intrinsic fluorescence this process follows first-order kinetics, with the rate constant for disassembly being proportional to the square of the H+ ion concentration, and with an activation energy of 155 kJ mol-1. A C-terminal deletion **mutant**, hEtxB214, similarly shows first-order kinetics for disassembly but with a higher pH threshold, resulting in disassembly being seen at pH 3.4 and below. These findings are consistent with the rate-limiting step for disassembly of human **EtxB** being the simultaneous disruption of two interfaces by protonation of two C-terminal carboxylates. By comparison, disassembly of the B-subunit of cholera toxin (CtxB), a protein which shows 80% sequence identity with **EtxB**, exhibits a much lower stability to acid conditions; with disassembly of CtxB occurring below pH 3.9, with an activation energy of 81 kJ mol-1. Reasons for the observed differences in acid stability are discussed, and the implications of these findings to

the development of oral vaccines using **EtxB** and CtxB are considered.

L25 ANSWER 15 OF 24 CAPLUS COPYRIGHT 2002 ACS
1995:404738 Document No. 122:209427 A pleiotropic secretion **mutant** of Aeromonas hydrophila is unable to secrete heterologously expressed E. coli enterotoxin: implication for common mechanisms of protein secretion. Yu, Jun; Hirst, Timothy R. (Res. Sch. of Biosciences, Univ. of Kent, Canterbury/Kent, CT2 7NJ, UK). Biochem. Soc. Trans., 23(1), 34S (English) 1995. CODEN: BCSTB5. ISSN: 0300-5127.
AB The exeE gene of A. hydrophila is assocd. with and operon (exe) previously predicted to encode the secretory machinery for aerolysin. Heterologous expression of the **etxB** gene of Escherichia coli (which encodes a heat-labile enterotoxin subunit) in wild-type and exeE gene insertion **mutants** of A. hydrophila showed that the wild-type but not the **mutant** organism secreted **EtxB** protein. The exeE gene of A. hydrophila is a homolog of the epsE gene of Vibrio cholerae. The protein encoded by the latter has significant homol. with the ExeE protein and gene epsE complements V. cholerae secretion **mutants**. Thus, data from the pleiotropic **mutant** support a common secretion mechanism for toxins from V. cholerae and A. hydrophila.

L25 ANSWER 16 OF 24 CAPLUS COPYRIGHT 2002 ACS
1994:597700 Document No. 121:197700 Assembly of Escherichia coli heat-labile enterotoxin and its secretion from Vibrio cholerae. Sandkvist, Maria; Overbye, Linda J.; Sixma, Titia K.; Hol, Wim G.J.; Bagdasarian, Michael (Unit. Lab. Animal. Med., Univ. Michigan, Ann Arbor, MI, USA). Dev. Plant Pathol., 3(Molecular Mechanisms of Bacterial Virulence), 293-309 (English) 1994. CODEN: DPPAEF.
AB A review with 64 refs. Subunits of the heat-labile enterotoxin of Escherichia coli (LT) assemble in the periplasm and are secreted through the outer membrane in Vibrio cholerae. Deletions or substitutions of residues at the carboxyl terminus of the B subunit (**EtxB**) result in **mutant** polypeptides that assemble into normal pentamers at 30.degree.C but cannot assemble at 42.degree.C in vivo. This defect may be suppressed by substitutions of single amino acid residues in regions that interact directly with the modified carboxyl terminus. Carboxyl terminal residues of **EtxB** thus appear to be required for formation or stabilization of an assembly intermediate of B subunit pentamerization but are not essential for the stability of the final pentamer. Secretion of the cholera toxin (CT) or of **EtxB** through the outer membrane of V. cholerae requires the functions of several genes that display extensive similarities to genes required for macromol. translocation in other Gram-neg. bacteria. One of the gene products required seems to be a cytoplasmic protein contg. ATP-binding domains. It may be a protein involved in the regulatory signal transduction.

L25 ANSWER 17 OF 24 MEDLINE DUPLICATE 13
95058206 Document Number: 95058206. PubMed ID: 7968540. Suppression of temperature-sensitive assembly **mutants** of heat-labile enterotoxin B subunits. Sandkvist M; Bagdasarian M. (Department of Microbiology, Michigan State University, East Lansing 48824.) MOLECULAR MICROBIOLOGY, (1993 Nov) 10 (3) 635-45. Journal code: 8712028. ISSN: 0950-382X. Pub. country: ENGLAND: United Kingdom. Language: English.
AB Deletions or substitutions of amino acids at the carboxyl-terminus of the heat-labile enterotoxin B subunit (**EtxB**) affect its assembly into pentamers in a temperature-dependent manner. At 42 degrees C, the mutations prevent the B subunits from achieving their final pentameric structure resulting in membrane association of the monomers. However, **mutant** B subunits produced at 30 degrees C assemble, in the periplasm, into pentamers that remain stable when transferred to 42 degrees C, indicating that the **mutant** pentamers are stable under

conditions where their formation is inhibited. The **mutant** pentamers are, similarly to wild-type pentamers, SDS-resistant and stable, in vitro, at temperatures up to 65 degrees C. This suggests that although the C-terminal amino acids are part of the subunit interface, they appear not to contribute significantly to the stability of the final pentameric complex, but are instead essential for the formation or stabilization of an assembly intermediate in the pentamerization process. Single second site mutations suppress the assembly defect of **mutant** EtxB191.5, which carries substitutions at its C-terminus. The Thr-->Ile replacement at position 75 in the alpha 2-helix probably restores the van der Waals contact between residues 75 and 101, which had been greatly reduced by the Met-->Leu substitution at position 101 in the beta 6-strand of EtxB191.5. Interaction between the alpha 2-helix and beta 6-strand which contains the C-terminus probably stabilizes a conformation essential for assembly and is therefore required for the formation of pentamers.

L25 ANSWER 18 OF 24 CAPLUS COPYRIGHT 2002 ACS
1993:424413 Document No. 119:24413 Analysis of enterotoxin synthesis in a Vibrio cholerae strain lacking DsbA, a periplasmic enzyme involved in disulfide bond formation. Findlay, Gordon; Yu, Jun; Hirst, Timothy R. (Biol. Lab., Univ. Kent, Canterbury/Kent, CT2 7NJ, UK). Biochem. Soc. Trans., 21(2), 212S (English) 1993. CODEN: BCSTB5. ISSN: 0300-5127.
AB To investigate the events of enterotoxin biogenesis the authors used a simplified system consisting of a vibrio strain with a chromosomal ctx gene deletion harboring the plasmid pMMB107, which encodes only the B-subunit of cholera-like enterotoxin (**EtxB**). Transposon (TnphoA) mutagenesis of this strain resulted in the identification of a **mutant**, UKC13::TnphoA.7A (pMMB107) with a 50-fold redn. in the level of the **EtxB** secretion. TnphoA insertion was found to be in a gene encoding a periplasmic protein with 40% homol. to the recently identified disulfide bond-forming protein (DsbA) of E. coli. To examine the role of DsbA in ExtB biogenesis, the dsbA::TnphoA **mutant** strain was cultured in minimal medium, pulse-labeled with 35S-Met and the fate of radiolabeled ExtB in periplasmic and medium fractions analyzed by SDS-PAGE and autoradiog. This demonstrated that **EtxB** was exported to the periplasm in both the **mutant** and the wild-type strain, but only secreted to the medium in the wild-type strain. The **EtxB** in the periplasm of the **mutant** strain was rapidly lost, probably as a result of proteolytic degrdn. This demonstrates that DsbA is not required for translocation of ExtB to the periplasm, but plays an important role in subsequent steps of toxin formation.

L25 ANSWER 19 OF 24 MEDLINE DUPLICATE 14
93101683 Document Number: 93101683. PubMed ID: 1465452. Intermolecular interactions between the A and B subunits of heat-labile enterotoxin from Escherichia coli promote holotoxin assembly and stability in vivo. Streatfield S J; Sandkvist M; Sixma T K; Bagdasarian M; Hol W G; Hirst T R. (Biological Laboratory, University of Kent, Canterbury, Great Britain.) PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (1992 Dec 15) 89 (24) 12140-4. Journal code: 7505876. ISSN: 0027-8424. Pub. country: United States. Language: English.
AB Cholera toxin and the related heat-labile enterotoxin (LT) produced by Escherichia coli consist of a holotoxin of one A subunit and five B subunits (AB5). Here we investigate the domains of the A subunit (EtxA) of E. coli LT which influence the events of B-subunit (**EtxB**) oligomerization and the formation of a stable AB5 holotoxin complex. We show that the C-terminal 14 amino acids of the A subunit comprise two functional domains that differentially affect oligomerization and holotoxin stability. Deletion of the last 14 amino acids (-14) from the A subunit resulted in a molecule that was significantly impaired in its capacity to promote the assembly of a **mutant** B subunit, EtxB191.5. In contrast, deletion of the last four amino acids (-4) from the A subunit gave a molecule that retained such a capacity. This suggests

that C-terminal residues within the -14 to -4 region of the A subunit are important for promoting the oligomerization of **EtxB**. In addition, we demonstrate that the truncated A subunit lacking the last 4 amino acids was unable to form a stable AB5 holotoxin complex even though it promoted B-subunit oligomerization. This suggests that the last 4 residues of the A subunit function as an "anchoring" sequence responsible for maintaining the stability of A/B subunit interaction during holotoxin assembly. These data represent an important example of how intermolecular interactions between polypeptides in vivo can modulate the folding and assembly of a macromolecular complex.

L25 ANSWER 20 OF 24 MEDLINE DUPLICATE 15

92374846 Document Number: 92374846. PubMed ID: 1324389. A homologue of the Escherichia coli DsbA protein involved in disulphide bond formation is required for enterotoxin biogenesis in Vibrio cholerae. Yu J; Webb H; Hirst T R. (Biological Laboratory, University of Kent, Canterbury, UK.) MOLECULAR MICROBIOLOGY, (1992 Jul) 6 (14) 1949-58. Journal code: 8712028. ISSN: 0950-382X. Pub. country: ENGLAND: United Kingdom. Language: English.

AB A strain of Vibrio cholerae, which had been engineered to express high levels of the non-toxic B subunit (**EtxB**) of Escherichia coli heat-labile enterotoxin, was subjected to transposon (TnphoA) mutagenesis. Two chromosomal TnphoA insertion mutations of the strain were isolated that showed a severe defect in the amount of **EtxB** produced. The loci disrupted by TnphoA in the two **mutant** derivatives were cloned and sequenced, and this revealed that the transposon had inserted at different sites in the same gene. The open reading frame of the gene predicts a 200-amino-acid exported protein, with a Cys-X-X-Cys motif characteristic of thioredoxin, protein disulphide isomerase, and DsbA (a periplasmic protein required for disulphide bond formation in E. coli). The V. cholerae protein exhibited 40% identity with the DsbA protein of E. coli, including 90% identity in the region of the active-site motif. Introduction of a plasmid encoding E. coli DsbA into the V. cholerae TnphoA derivatives was found to restore enterotoxin formation, whilst expression of Etx or **EtxB** in a dsbA **mutant** of E. coli confirmed that DsbA is required for enterotoxin formation in E. coli. These results suggest that, since each **EtxB** subunit contains a single intramolecular disulphide bond, a transient intermolecular interaction with DsbA occurs during toxin subunit folding which catalyses formation of the disulphide in vivo.

L25 ANSWER 21 OF 24 MEDLINE DUPLICATE 16

92268852 Document Number: 92268852. PubMed ID: 1588306. Expression of the B subunit of Escherichia coli heat-labile enterotoxin in a marine Vibrio and in a **mutant** that is pleiotropically defective in the secretion of extracellular proteins. Leece R; Hirst T R. (Department of Genetics, University of Leicester, UK.) JOURNAL OF GENERAL MICROBIOLOGY, (1992 Apr) 138 (Pt 4) 719-24. Journal code: 0375371. ISSN: 0022-1287. Pub. country: ENGLAND: United Kingdom. Language: English.

AB A marine Vibrio (designated Vibrio sp. 60) that is related to Vibrio anguillarum was used as a host for a plasmid that encodes the non-toxic B subunit (**EtxB**) of Escherichia coli heat-labile enterotoxin. Expression of **EtxB** in Vibrio sp. 60 resulted in the efficient and selective secretion of the B subunit into the extracellular growth medium. This indicated that Vibrio sp. 60, which does not normally produce cholera-like enterotoxins, nonetheless possesses a secretory machinery that permits these toxins to be translocated across its cytoplasmic and outer membranes. Expression of **EtxB** in a sec **mutant** of Vibrio sp. 60 (MVT1192), which had previously been shown to be defective in the secretion of several extracellular proteins, resulted in approximately 95% of the B subunit remaining entrapped within the periplasm of the bacterial cell envelope. This implies that the mutation in MVT1192 defines a locus that determines a common step in the secretion of extracellular proteins, including oligomeric toxins.

L25 ANSWER 22 OF 24 MEDLINE DUPLICATE 17

92140031 Document Number: 92140031. PubMed ID: 1779757. Targeting and assembly of an oligomeric bacterial enterotoxoid in the endoplasmic reticulum of Saccharomyces cerevisiae. Schonberger O; Hirst T R; Pines O. (Department of Molecular Biology, Hebrew University, Hadassah Medical School, Jerusalem, Israel.) MOLECULAR MICROBIOLOGY, (1991 Nov) 5 (11) 2663-71. Journal code: 8712028. ISSN: 0950-382X. Pub. country: ENGLAND: United Kingdom. Language: English.

AB A hybrid protein consisting of the Escherichia coli lipoprotein signal sequence attached to the mature sequence of the B subunit of heat-labile enterotoxin (Lipo-**EtxB**) was expressed in yeast and E. coli. Analyses of cell lysates from Saccharomyces cerevisiae and E. coli expressing the protein revealed that both organisms were able to assemble Lipo-**EtxB** into oligomers that were (i) stable in the presence of sodium dodecyl sulphate, (ii) resistant to proteinase K degradation, and (iii) able to bind to GM1-ganglioside receptors. Each of these properties are characteristic of the wild-type B subunit pentamer produced in E. coli. Assembly of Lipo-**EtxB** was found to be unaffected in a sec18 **mutant** of S. cerevisiae, which possesses a temperature-sensitive defect in protein transport from the endoplasmic reticulum (ER) to the Golgi apparatus, but was found not to assemble in a sec53 **mutant**, which causes the misfolding of proteins targeted to the ER. A kar2-1 mutation with a defect in the yeast homologue of BiP caused an 18-fold reduction in Lipo-**EtxB** assembly at the non-permissive temperature in S. cerevisiae. However, introduction of the wild-type KAR2 gene on a plasmid into the kar2-1 **mutant** completely suppressed the inhibition of Lipo-**EtxB** assembly. This provides the first evidence that KAR2 facilitates the assembly of an oligomeric protein in yeast and thus implicates KAR2 as a 'molecular chaperone'. The possible mechanisms of enterotoxoid assembly in E. coli and S. cerevisiae are discussed.

L25 ANSWER 23 OF 24 MEDLINE DUPLICATE 18

90368708 Document Number: 90368708. PubMed ID: 2203772. Minimal deletion of amino acids from the carboxyl terminus of the B subunit of heat-labile enterotoxin causes defects in its assembly and release from the cytoplasmic membrane of Escherichia coli. Sandkvist M; Hirst T R; Bagdasarian M. (Department of Microbiology, Michigan State University, Lansing 48909.) JOURNAL OF BIOLOGICAL CHEMISTRY, (1990 Sep 5) 265 (25) 15239-44. Journal code: 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.

AB Minimal alterations at the carboxyl terminus of the B subunit (**EtxB**) of heat-labile enterotoxin from Escherichia coli were found to have a marked effect on the assembly and release of this polypeptide into the periplasm. Nine **mutant EtxB** polypeptides were obtained by genetic manipulation of the 3'-end of the **etxB** gene using Bal31 nuclease digestion and codon substitution. A correlation was observed between the magnitude of the changes introduced at the carboxyl terminus and the extent to which the **mutant** polypeptides were defective in assembly and release. Some of the **mutant** B subunits, exemplified by those in which the last 2 amino acids had been deleted or in which the last 4 residues had been replaced by three different ones, were found to be only partially defective, with a proportion being associated with the periplasmic face of the cytoplasmic membrane and the remainder being exported to the periplasm. The portion associated with membranes was detected as monomers on sodium dodecyl sulfate-polyacrylamide gels, whereas the portion exported to the periplasm were detected as assembled oligomers. We conclude that the last few amino acids at the carboxyl terminus of **EtxB** exert a profound influence on the assembly and release of the B subunit from the cytoplasmic membrane during export in E. coli.

L25 ANSWER 24 OF 24 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
1987:488218 Document No.: BA84:122861. ALTERATIONS AT THE CARBOXYL TERMINUS CHANGE ASSEMBLY AND SECRETION PROPERTIES OF THE B SUBUNIT OF ESCHERICHIA-COLI HEAT-LABILE ENTEROTOXIN. SANDKVIST M; HIRST T R; BAGDASARIAN M. DEP. GENET., UNIV. LEICESTER, LEICESTER LE1 7RH, ENGLAND.. J BACTERIOL, (1987) 169 (10), 4570-4576. CODEN: JOBAAY. ISSN: 0021-9193. Language: English.

AB The gene encoding the B subunit of heat-labile enterotoxin (**etxB**) was mutated at its 3' end by targeted addition of random nucleotide sequences. Gene products from five mutated **etxB** genes, all of which were shown to encode B subunits with short carboxy-terminal amino acid extensions, were analyzed with respect to a range of functional and structural properties. One class of altered B subunits, exemplified by EtxB124 and EtxB138, which both have seven extra amino acid residues, were found to be specifically defective in their ability to stably associate with A subunits and form holotoxin. Other altered B subunits were less subtlety affected by extensions at their C termini and were, in addition to their failure to associate with A subunits, unable to translocate into the periplasm of Escherichia coli, to pentamerize, or to bind to GM1 ganglioside. This suggests that the carboxy-terminal domain of **EtxB** mediates A subunit-B subunit interaction.

=> s asthma
L26 260989 ASTHMA

=> s l26 and treatment
L27 58201 L26 AND TREATMENT

=> s l27 adn cholera toxin
MISSING OPERATOR L27 ADN
The search profile that was entered contains terms or
nested terms that are not separated by a logical operator.

=> s l27 and cholera toxin
L28 22 L27 AND CHOLERA TOXIN

=> dup remove l28
PROCESSING COMPLETED FOR L28
L29 13 DUP REMOVE L28 (9 DUPLICATES REMOVED)

=> d l29 1-13 cbib abs

L29 ANSWER 1 OF 13 CAPLUS COPYRIGHT 2002 ACS
2002:272796 Document No. 136:293510 Anti-allergic vaccines comprising peptides of Fc portion of IgE .epsilon. heavy chain and carrier protein. Morsey, Mohamad Ali; Sheppard, Michael George; Wheeler, David Walter (Pfizer Products Inc., USA). Eur. Pat. Appl. EP 1195161 A2 20020410, 38 pp. DESIGNATED STATES: R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO. (English). CODEN: EPXXDW. APPLICATION: EP 2001-307247 20010824. PRIORITY: US 2000-PV228989 20000830.

AB The present invention provides compns. and methods for the use of antigenic peptides derived from the Fc portion of the epsilon heavy chain of an IgE mol. as vaccines for the **treatment** and prevention of IgE-mediated allergic disorders. In particular, the invention provides compns., methods for the **treatment** and prevention of IgE-mediated allergic disorders comprising an immunogenic amt. of one or more antigenic peptides derived from the CH3 domain or junction of Ch-3/CH4 domain of an IgE mol. and methods for the evaluation of IgE mediated allergies in dogs. The allergic disorder is **asthma**, allergic rhinitis, gastrointestinal allergy, food allergy, eosinophilia, conjunctivitis, or glomerular nephritis. The vaccine compns. may also

comprises carrier protein such as KLH, PhoE, rmLT, TraT and gD from BhV-1 virus; and adjuvant such as aluminum hydroxide, monophosphoryl lipid A, Thr-MDP, immunostimulatory oligonucleotide, cytokine, interleukin 12, interleukin 2, interleukin 1, saponin, **cholera toxin**, heat labile toxin, etc.

L29 ANSWER 2 OF 13 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.
2002069171 EMBASE Induction of mucosal tolerance to Bet v 1, the major birch pollen allergen - A review. Wiedermann U.; Kraft D.. Prof. U. Wiedermann, Department of Pathophysiology, University Vienna, Waehringer Guertel 18-20, A-1090 Vienna, Austria. ursula.wiedermann@akh-wien.ac.at. Allergy and Clinical Immunology International 14/1 (17-24) 2002.
Refs: 99.
ISSN: 0838-1925. CODEN: ACIIFH. Pub. Country: Switzerland. Language: English. Summary Language: English.
AB Background: The major birch pollen allergen Bet v 1 is one of the most prevalent environmental allergens responsible for allergic airway inflammation in man. Mucosal application of soluble antigens leads to a state of antigen-specific, systemic nonresponsiveness, termed oral/mucosal tolerance. Within recent years there has been increasing interest in mucosal tolerance induction to be used as a **treatment** strategy against immunological disorders, including type I allergies. Methods/data base: A mouse model of allergic **asthma** was used to study the effectiveness of mucosal tolerance induction with recombinant Bet v 1 and hypoallergenic molecules thereof. In addition certain mucosal adjuvants/mucosal antigen delivery systems in conjunction with the allergen were used to prevent or treat type I allergic immune responses to birch pollen and its major allergen Bet v 1. Results: Inhalation of birch pollen antigen in conjunction with the mucosal adjuvant **cholera-toxin** induced a Th1-like response in naive animals and modulated an already established allergic immune response. Intranasal application of Bet v 1 conjugated to the nontoxic **cholera toxin** B (CTB)-subunit enhanced subsequent allergic sensitization, whereas CTB coupled to the dietary allergen ovalbumin reduced antigen-specific immunoglobulin E (IgE) production, indicating that the effects of the mucosal antigen delivery system depended on the nature of the coupled allergen. Mucosal (nasal and oral) administration of unconjugated Bet v 1 or hypoallergenic derivatives thereof could inhibit/suppress allergic sensitization and airway inflammation in naive and in sensitized animals. Conclusion: We conclude from our studies that mucosal tolerance induction with recombinant allergens and their hypoallergenic derivatives - with or without the use of mucosal adjuvants - could provide a safe and convenient alternative **treatment** to conventional immunotherapy.

L29 ANSWER 3 OF 13 SCISEARCH COPYRIGHT 2002 ISI (R)
2001:868781 The Genuine Article (R) Number: 485NK. Food Allergy Herbal Formula-1 (FAHF-1) blocks peanut-induced anaphylaxis in a murine model. Li X M (Reprint); Zhang M F; Huang C K; Srivastava K; Teper A A; Zhang L B; Schofield B H; Sampson H A. Mt Sinai Sch Med, Dept Pediat, 1 Gustave L Levy Pl, New York, NY 10029 USA (Reprint); Mt Sinai Sch Med, Dept Pediat, New York, NY 10029 USA; Mt Sinai Sch Med, Ctr Comparat Med & Surg, New York, NY 10029 USA; Johns Hopkins Univ, Bloomberg Sch Publ Hlth, Dept Environm Hlth Sci, Baltimore, MD USA. JOURNAL OF ALLERGY AND CLINICAL IMMUNOLOGY (OCT 2001) Vol. 108, No. 4, pp. 639-646. Publisher: MOSBY, INC. 11830 WESTLINE INDUSTRIAL DR, ST LOUIS, MO 63146-3318 USA. ISSN: 0091-6749 . Pub. country: USA. Language: English.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS
AB Background: Peanut allergy is a major cause of fatal and near-fatal anaphylactic reactions to foods. There is no curative therapy for this condition. Traditional Chinese medicines have been reported to have antiallergic properties, which might be useful for treating peanut allergy.
Objective: The purpose of this study was to investigate the effects of

a Chinese herbal formula, FAHF-1, on peanut anaphylactic reactions in a mouse model of peanut allergy.

Methods: Mice were sensitized with freshly ground whole peanut in the presence of **cholera toxin** and boosted 1 and 3 weeks later. FAHF-1 **treatment** was initiated 1 week later and continued for 7 weeks. After **treatment**, mice were challenged with peanut, and anaphylactic symptoms, body temperatures, and plasma histamine and IgE levels were measured. T-cell proliferative responses and cytokine production were also determined.

Results: FAHF-1 completely blocked peanut-induced anaphylactic symptoms and markedly reduced mast cell degranulation and histamine release. Peanut-specific serum IgE levels were significantly reduced by 2 weeks of **treatment** at the time of challenge, and they remained lower 4 weeks after discontinuation of **treatment**. FAHF-1 significantly reduced peanut-induced lymphocyte proliferation is well as IL-4, IL-5, and IL-13 synthesis but not IFN-gamma synthesis. No toxic effects on liver or kidney functions were observed, nor was there any overall immune suppression.

Conclusion: FAHF-1 protected peanut-sensitized mice from anaphylactic reactions and significantly reversed established IgE-niediated peanut allergy. This suggests that FAHF-1 might prove valuable for the **treatment** of peanut allergy.

L29 ANSWER 4 OF 13 MEDLINE DUPLICATE 1

2001478843 Document Number: 21413478. PubMed ID: 11522597. Suppression of granulocyte/macrophage colony-stimulating factor release from human monocytes by cyclic AMP-elevating drugs: role of interleukin-10. Seldon P M; Giembycz M A. (Thoracic Medicine, National Heart and Lung Institute, Imperial College School of Medicine, London SW3 6LY, UK.) BRITISH JOURNAL OF PHARMACOLOGY, (2001 Sep) 134 (1) 58-67. Journal code: 7502536. ISSN: 0007-1188. Pub. country: England: United Kingdom. Language: English.

AB 1. Granulocyte/macrophage colony-stimulating factor (GM-CSF) is a pro-inflammatory cytokine secreted by cells of the monocyte/macrophage lineage and has been implicated in the pathogenesis of bronchitis and **asthma**. 2. In the present study we have evaluated the effect of several cyclic AMP-elevating agents on lipopolysaccharide (LPS)-induced GM-CSF release from human monocytes and the extent to which the anti-inflammatory cytokine, interleukin (IL)-10, is involved. 3. LPS evoked a concentration-dependent generation of GM-CSF from human monocytes that was inhibited, at the mRNA and protein level, by 8-Br-cyclic AMP, **cholera toxin**, prostaglandin E2 (PGE2) and a number of structurally dissimilar phosphodiesterase (PDE) 4 inhibitors. 4. Pre-**treatment** of monocytes with a concentration of an anti-IL-10 monoclonal antibody that abolished the inhibitory action of a maximally effective concentration of exogenous human recombinant IL-10, significantly augmented LPS-induced GM-CSF generation. This effect was associated with a parallel upwards displacement of the concentration-response curves that described the inhibition of GM-CSF by PGE2, 8-Br-cyclic AMP and the PDE4 inhibitor, rolipram, without significantly changing the potency of any drug. Consequently, the maximum percentage inhibition of GM-CSF release was reduced. Further experiments established that the reduction in the maximum inhibition of GM-CSF release seen in anti-IL-10-treated cells was not due to functional antagonism as rolipram, PGE2 and 8-Br-cyclic AMP were equi-effective at all concentrations of LPS studied. 5. These data indicate that cyclic AMP-elevating drugs attenuate the elaboration of GM-CSF from LPS-stimulated human monocytes by a mechanism that is not mediated via IL-10. Suppression of GM-CSF from monocytes may explain, at least in part, the efficacy of PDE4 inhibitors in clinical trials of chronic obstructive pulmonary disease.

L29 ANSWER 5 OF 13 SCISEARCH COPYRIGHT 2002 ISI (R)

2000:58681 The Genuine Article (R) Number: 273LF. Therapeutic potential of

phosphodiesterase-4 and-3 inhibitors in Th1-mediated autoimmune diseases. Bielekova B; Lincoln A; McFarland H; Martin R (Reprint). NINCDS, NEUROIMMUNOL BRANCH, NIH, BLDG 10, ROOM 5B-16, 10 CTR DR MSC 1400, BETHESDA, MD 20892 (Reprint); NINCDS, NEUROIMMUNOL BRANCH, NIH, BETHESDA, MD 20892. JOURNAL OF IMMUNOLOGY (15 JAN 2000) Vol. 164, No. 2, pp. 1117-1124. Publisher: AMER ASSOC IMMUNOLOGISTS. 9650 ROCKVILLE PIKE, BETHESDA, MD 20814. ISSN: 0022-1767. Pub. country: USA. Language: English. *ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS*

AB Phosphodiesterase-4 (PDE4) inhibitors have the potential to modulate immune responses from the Th1 toward the Th2 phenotype and are considered candidate therapies for Th1-mediated autoimmune disorders. However, depending on the model and cell types employed, studies of atopic individuals have come to the opposite conclusion, i.e., that PDE inhibitors may be beneficial in **asthma**, Using in vitro immunopharmacologic techniques we analyzed the effects of PDE4 and PDE3 inhibitors on human immune cells to address these discrepancies and broaden our understanding of their mechanism of action. Our results indicate that PDE inhibitors have complex inhibitory effects within in vivo achievable concentration ranges on Th1-mediated immunity, whereas Th2-mediated responses are mostly unaffected or enhanced. The Th2 skewing of the developing immune response is explained by the effects of PDE inhibitors on several factors contributing to T cell priming: the cytokine milieu; the type of costimulatory signal, i.e., up-regulation of CD86 and down-regulation of CD80; and the Ag avidity. The combination of PDE4 and PDE3 inhibitors expresses synergistic effects and may broaden the therapeutic window. Finally, we observed a differential sensitivity to PDE inhibition in autoreactive vs foreign Ag-specific T cells and cells derived from multiple sclerosis patients vs those derived from healthy donors. This suggests that PDE inhibition,weakens the strength of the T cell stimulus and corrects the underlying disease-associated cytokine skew in T cell-mediated autoimmune disorders. These new findings broaden the understanding of the immunomodulatory actions of PDE inhibitors and underscore their promising drug profile for the **treatment** of autoimmune disorders.

L29 ANSWER 6 OF 13 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.DUPLICATE 2

2000239863 EMBASE Albuterol-induced downregulation of Gs.alpha. accounts for pulmonary .beta.2- adrenoceptor desensitization in vivo. Finney P.A.; Belvisi M.G.; Donnelly L.E.; Chuang T.-T.; Mak J.C.W.; Scorer C.; Barnes P.J.; Adcock I.M.; Giembycz M.A.. M.A. Giembycz, Department of Thoracic Medicine, Imperial College School of Medicine, National Heart and Lung Institute, Dovehouse Street, London SW3 6LY, United Kingdom. m.giembycz@ic.ac.uk. Journal of Clinical Investigation 106/1 (125-135) 2000.

Refs: 48.

ISSN: 0021-9738. CODEN: JCINAO. Pub. Country: United States. Language: English. Summary Language: English.

AB The aim of the present study was to develop a chronic in vivo model of pulmonary .beta.2-adrenoceptor desensitization and to elucidate the nature and molecular basis of this state. Subcutaneous infusion of rats with albuterol for 7 days compromised the ability of albuterol, given acutely, to protect against acetylcholine-induced bronchoconstriction. The bronchoprotective effect of prostaglandin E2, but not forskolin, was also impaired, indicating that the desensitization was heterologous and that the primary defect in signaling was upstream of adenylyl cyclase. .beta.2-Adrenoceptor density was reduced in lung membranes harvested from albuterol-treated animals, and this was associated with impaired albuterol-induced cyclic adenosine monophosphate (cAMP) accumulation and activation of cAMP-dependent protein kinase ex vivo. Gs.alpha. expression was reduced in the lung and tracheae of albuterol-treated rats, and **cholera toxin**-induced cAMP accumulation was blunted.
Chronic **treatment** of rats with albuterol also increased cAMP phosphodiesterase activity and G protein-coupled receptor kinase-2, but

the extent to which these events contributed to .beta.2-adrenoceptor desensitization was unclear given that forskolin was active in both groups of animals and that desensitization was heterologous. Collectively, these results indicate that albuterol effects heterologous desensitization of pulmonary Gs-coupled receptors in this model, with downregulation of Gs.alpha. representing a primary molecular etiology.

L29 ANSWER 7 OF 13 SCISEARCH COPYRIGHT 2002 ISI (R)
1998:502012 The Genuine Article (R) Number: ZW326. In vivo anti-influenza virus activity of Kampo (Japanese herbal) medicine ''Sho-seiryu-to'' - Stimulation of mucosal immune system and effect on allergic pulmonary inflammation model mice. Nagai T (Reprint); Yamada H. KITASATO INST, ORIENTAL MED RES CTR, MINATO KU, 5-9-1 SHIROKANE, TOKYO 108, JAPAN (Reprint). IMMUNOPHARMACOLOGY AND IMMUNOTOXICOLOGY (AUG 1998) Vol. 20, No. 2, pp. 267-281. Publisher: MARCEL DEKKER INC. 270 MADISON AVE, NEW YORK, NY 10016. ISSN: 0892-3973. Pub. country: JAPAN. Language: English. *ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS*

AB When BALB/c mice were treated with a Kampo (Japanese herbal) medicine ''Sho-seiryu-to (SST)'' (1 g/kg, 10 times) orally from 7 days before to 5 days after the infection and infected with mouse-adapted influenza virus A/PR/8/34 by nasal-site restricted infection, SST caused increment of the influenza virus hemagglutinin-specific IgA antibody secreting cells in nasal lymphocyte but not in Peyer's patch lymphocyte at 6 days after infection in comparison with water-treated mice. Oral administration of SST also augmented IL-2 receptor beta chain(+) (activated) T-cell in Peyer's patch lymphocyte, but not in the nasal lymphocyte. We previously reported that SST showed potent anti-influenza virus activity through augmentation of the antiviral IgA antibody titer in the nasal and broncho-alveolar cavities of the mice (T. Nagai and Il. Yamada, 1994, Int. J. Immunopharmacol. 16, 605-613). These results suggest that oral administration of SST shows anti-influenza virus activity in the nasal cavity by activatation of T-cell in Peyer's patch lymphocyte and stimulation of production of anti-influenza virus IgA antibody in nasal lymphocyte. When ovalbumin-sensitized allergic pulmonary inflammation model mice were administered orally with SST(I g/kg) from 8 days before(ll times) or from 2 h after (4 times) to 4 days after the infection and infected with mouse-adapted influenza virus A/PR/8/34, replications of the virus in the both nasal and broncho-alveolar cavities or only nasal cavity were significantly inhibited at 5 days after infection in comparison with water-treated control by augmenting antiviral IgA antibody, respectively. These results suggest that SST is useful for both prophylaxis and **treatment** of influenza virus infection on patients with allergic pulmonary inflammation, such as bronchial **asthma**.

L29 ANSWER 8 OF 13 MEDLINE DUPLICATE 3
95173441 Document Number: 95173441. PubMed ID: 7532667. Activation of cAMP-dependent pathways in human airway smooth muscle cells inhibits TNF-alpha-induced ICAM-1 and VCAM-1 expression and T lymphocyte adhesion. Panettieri R A Jr; Lazaar A L; Pure E; Albelda S M. (Department of Medicine, University of Pennsylvania Medical Center, Philadelphia.) JOURNAL OF IMMUNOLOGY, (1995 Mar 1) 154 (5) 2358-65. Journal code: 2985117R. ISSN: 0022-1767. Pub. country: United States. Language: English.

AB **Asthma** is a disease of airway inflammation and hyper-reactivity associated with lymphocytic infiltration in the bronchial submucosa. We recently demonstrated that human airway smooth muscle (ASM) cells express the cell adhesion molecules ICAM-1 and VCAM-1, which are up-regulated by cytokines such as TNF-alpha, and which mediate binding of activated T lymphocytes. In this study, we examined whether an increase in [cAMP]i, presumably via activation of cAMP-dependent protein kinase, modulates TNF-alpha-induced ICAM-1 and VCAM-1 on ASM. We found that **treatment** of ASM with either forskolin, which directly activates adenylyl cyclase, or with **cholera toxin**, which activates the heterotrimeric GTP-binding protein, Gs, inhibited

TNF-alpha-induced cell adhesion molecule expression. In addition, **treatment** with either isoproterenol or prostaglandin E2, which activates receptors coupled to Gs and increases [cAMP]i, also inhibited TNF-alpha-induced expression of ICAM-1 and VCAM-1 on ASM. Furthermore, adhesion of activated T cells to TNF-alpha-stimulated ASM was inhibited by treating the ASM cells with either forskolin or PGE2. These data suggest that cAMP-dependent protein kinase activation decreases cytokine-induced expression of cell adhesion molecules on ASM cells, modulates T cell binding to airway myocytes and, thus, suggests novel therapeutic approaches to airway inflammation.

L29 ANSWER 9 OF 13 SCISEARCH COPYRIGHT 2002 ISI (R)
95:739758 The Genuine Article (R) Number: TA567. SUPPRESSION OF LIPOPOLYSACCHARIDE-INDUCED TUMOR-NECROSIS-FACTOR-ALPHA GENERATION FROM HUMAN PERIPHERAL-BLOOD MONOCYTES BY INHIBITORS OF PHOSPHODIESTERASE-4 - INTERACTION WITH STIMULANTS OF ADENYLYL-CYCLASE. SELDON P M; BARNES P J; MEJA K; GIEMBYCZ M A (Reprint). ROYAL BROMPTON NATL HEART & LUNG INST, DEPT THORAC MED, DOVEHOUSE ST, LONDON SW3 6LY, ENGLAND (Reprint); ROYAL BROMPTON NATL HEART & LUNG INST, DEPT THORAC MED, LONDON SW3 6LY, ENGLAND. MOLECULAR PHARMACOLOGY (OCT 1995) Vol. 48, No. 4, pp. 747-757. ISSN: 0026-895X. Pub. country: ENGLAND. Language: ENGLISH.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB We assessed the role of cyclic nucleotides in modulating lipopolysaccharide (LPS)-induced tumor necrosis factor-alpha (TNF-alpha) generation in human peripheral blood monocytes. Exposure of monocytes to LPS (3 ng/ml) evoked a delayed, time-dependent generation of TNF-alpha that reached a maximum level 5-6 hr after LPS challenge and remained constant for up to 24 hr. This effect was concentration dependent and resulted in a 20-40-fold increase in the release of TNF-alpha that was sensitive to actinomycin D and cycloheximide. **Treatment** of monocytes with agents reputed to activate the cAMP/cAMP-dependent protein kinase (PKA) cascade in general inhibited LPS-induced TNF-alpha generation. Thus, the beta(2)-adrenoceptor agonists albuterol and procaterol partially (similar to 40%) suppressed TNF-alpha generation in a propranolol-sensitive manner. Furthermore, 8-bromo-cAMP, **cholera toxin**, prostaglandin E(2), and a number of drugs (i.e., rolipram (ZK 62711), denbufylline (BRL 30892), Ro 20-1724, benafentrine (AH 21-132), that inhibit the phosphodiesterase (PDE) 4 isoenzyme family abolished cytokine generation. In contrast, forskolin, inhibitors of PDE3 and PDE5, and activators of soluble and particulate guanylyl cyclase were essentially inactive. Interestingly, rolipram failed to potentiate the inhibitory effect of albuterol on LPS-induced TNF-alpha biosynthesis but, paradoxically, synergized with albuterol in the generation of cAMP and in the activation of PKA. When PGE(2) was used to activate adenylyl cyclase, however, rolipram potentiated cAMP accumulation, PKA activation, and inhibition of TNF-alpha generation. In contrast, forskolin did not increase the cAMP content of monocytes in the absence or presence of rolipram. Collectively, these data suggest that LPS-induced TNF-alpha generation by human peripheral blood monocytes is due to increased transcription and subsequent translation of the TNF-alpha gene and that these effects are suppressed by a range of agents that activate the cAMP/PKA cascade. However, the failure of rolipram to potentiate the inhibitory effect of albuterol and procaterol on TNF-alpha generation suggests that beta(2)-adrenoceptor agonists may affect gene expression and/or post-transcriptional regulatory processes by, at least in part, a cAMP-independent mechanism(s).

L29 ANSWER 10 OF 13 SCISEARCH COPYRIGHT 2002 ISI (R)
95:69788 The Genuine Article (R) Number: QB189. 3-ISOBUTYL-1-METHYLXANTHINE INCREASES ALPHA-1-ADRENERGIC RECEPTOR SENSITIVITY AND DENSITY IN DDT1-MF2 SMOOTH-MUSCLE CELLS. SCHACHTER J B; WOLFE B B (Reprint). GEORGETOWN UNIV, SCH MED, DEPT PHARMACOL, 3900 RESERVOIR RD NW, WASHINGTON, DC, 20007 (Reprint); GEORGETOWN UNIV, SCH MED, DEPT PHARMACOL, WASHINGTON, DC, 20007

. JOURNAL OF PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS (JAN 1995) Vol. 272, No. 1, pp. 215-223. ISSN: 0022-3565. Pub. country: USA. Language: ENGLISH.

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB The effect of chronic exposure of DDT1-MF2 smooth muscle cells to the phosphodiesterase inhibitor 3-isobutyl-1-methylxanthine (IBMX) was investigated with regard to the dynamics of alpha-1-adrenergic receptors. After 48 hr of exposure to 750 mu M IBMX, the magnitude of the maximal phospholipase C response to norepinephrine was increased approximately 2-fold and the potency of norepinephrine was increased almost 3-fold. Similar effects were noted for the response to ATP. The density of alpha-1-adrenergic receptors, as defined by [H-3]prazosin binding to membranes was increased 2-fold. In addition, chronic **treatment** with IBMX prevented agonist-induced desensitization of alpha-1-adrenergic receptors and enhanced the rate of receptor resensitization subsequent to desensitization by a combination of agonist and phorbol ester. These effects appear to be regulated by a cyclic AMP-dependent mechanism. Thus, chronic exposure of smooth muscle cells to phosphodiesterase inhibition may activate compensatory mechanisms that lead to enhanced sensitivity to contractile stimuli. The potential importance of such compensatory mechanisms in the **treatment** and etiology of smooth muscle dysfunction is briefly discussed.

L29 ANSWER 11 OF 13 MEDLINE

96288711 Document Number: 96288711. PubMed ID: 8752494. Involvement of G proteins between receptors and KCa channels in the regulation of airway tone by the autonomic nervous system. Kume H; Takagi K. (Second Department of Internal Medicine, School of Medicine, Nagoya University, Japan.) NIHON KYOBU SHIKKAN GAKKAI ZASSHI. JAPANESE JOURNAL OF THORACIC DISEASES, (1995 Dec) 33 Suppl 116-24. Journal code: 7505737. ISSN: 0301-1542. Pub. country: Japan. Language: Japanese.

AB The mechanical tone of the airways is regulated by the autonomic nervous system, partly via the activity of ion channels. Ca(2+)-activated K+ (KCa) channels are densely distributed on tracheal smooth muscle cells. We found that beta-adrenergic agonists can augment KCa channel activity via the alpha subunit of the stimulatory GTP-binding (G) protein of adenylyl cyclase, Gs, linked with beta-receptors, and that muscarinic agonists can suppress the activity of this channel via the inhibitory G protein of adenylyl cyclase (pertussis toxin-sensitive G protein), Gi, linked with muscarinic receptors. These results show that there is a dual regulation system of KCa channels, which involves stimulation of the two receptors. Records of isometric tension from guinea pig tracheas incubated with pertussis toxin and **cholera toxin** show that regulation of KCa channels mediated by Gi and Gs may be important in the mechanical antagonism by the two receptor agonists, and they show that G proteins coupling between receptors and KCa channels may be important in beta-adrenergic bronchodilation in the **treatment** of **asthma**. In a previous study in eight atopic asthmatic patients, pretreatment with a beta-agonist abolished allergen-induced bronchoconstriction with no increment in mean plasma histamine, results that are similar to those obtained with cromyolyn sodium, a membrane stabilizer. The membrane-delimited reaction may be a key process in the autonomic regulation of airway tone. In immediate asthmatic reactions (IAR), histamine release from mast cells, contraction of airway smooth muscle, and transmitter release from post-ganglionic neurons within parasympathetic ganglia are believed to be caused by membrane hypopolarization. Because activation of KCa channels leads to hyperpolarization, beta-agonists that cause membrane hyperpolarization (short acting beta-agonists) may antagonize IAR at the level of the cell membrane. In late asthmatic reactions (LAR), short-acting beta-agonists do not have marked effects. However, recent reports have indicated that long-acting beta-agonists that do not cause hyperpolarization can inhibit LAR. Cromakalim, an ATP-sensitive K+ channel activator, reduces the

"morning dip" when it is given orally to patients with nocturnal **asthma**. These findings show that activation of K+ channels may be useful in therapy of bronchial **asthma**.

L29 ANSWER 12 OF 13 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.
92331572 EMBASE Document No.: 1992331572. New drugs for **asthma**.
Barnes P.J.. Dept. of Thoracic Medicine, National Heart and Lung Institute, Dovehouse St.,London SW3 6LY, United Kingdom. European Respiratory Journal 5/9 (1126-1136) 1992.
ISSN: 0903-1936. CODEN: ERJOEI. Pub. Country: Denmark. Language: English. Summary Language: English.
AB Several new drugs are now under development for the **treatment** of **asthma**, either as improvements to existing classes of therapy or as novel agents. Amongst bronchodilators, long-acting inhaled .beta.2-agonists (salmeterol and formoterol) look very promising and there is also interest in selective phosphodiesterase inhibitors, K+ channel-openers and nitrodilators. There are several new inhaled corticosteroids under development and more selective agents include leukotriene antagonists, 5-lipoxygenase inhibitors, bradykinin and tachykinin antagonists and immunomodulators. In the future, adhesion molecule inhibitors and cytokine inhibitors may be developed.

L29 ANSWER 13 OF 13 CAPLUS COPYRIGHT 2002 ACS
1991:115084 Document No. 114:115084 Antiallergy agents containing allergens and adjuvants and antiallergy agents containing allergen-adjuvant complexes. Watanabe, Naohiro (Japan). Jpn. Kokai Tokkyo Koho JP 02235823 A2 19900918 Heisei, 5 pp. (Japanese). CODEN: JKXXAF. APPLICATION: JP 1989-54887 19890309.
AB Antiallergy agents, which are useful for **treatment** of type I allergy, e.g. **asthma** and allergic rhinitis, and have low toxicity, contain (1) allergens and adjuvants stimulating prodn. of IgA against the allergens; or (2) allergens bonded with the adjuvants (via spacers). Nasal administration of 10 .mu.g ovalbumin and 1.0 .mu.g **cholera toxin** produced antiovalbumin IgA in mice, vs. none, without the toxin.

=> d his

(FILE 'HOME' ENTERED AT 09:31:40 ON 01 JUL 2002)

FILE 'MEDLINE, EMBASE, BIOSIS, SCISEARCH, CAPLUS' ENTERED AT 09:32:05 ON 01 JUL 2002

L1	51040	S GANGLIOSIDE
L2	2708	S L1 AND MODULAT?
L3	219	S L2 AND AGENT
L4	105	S L3 AND TREATMENT
L5	1	S L4 AND ALLERGY
L6	6	S L4 AND HYPERSENSITIVITY
L7	2	DUP REMOVE L6 (4 DUPLICATES REMOVED)
L8	766	S "ETX"
L9	8	S L8 AND GANGLIOSIDE
L10	4	DUP REMOVE L9 (4 DUPLICATES REMOVED)
L11	9952	S "CTX"
L12	64	S L11 AND GANGLIOSIDE
L13	0	S L12 AND HYPERSENSITIVITY
L14	0	S L12 AND IGE
L15	0	S L12 AND ALLERGY
L16	1	S L12 AND MUTANT
L17	20	DUP REMOVE L12 (44 DUPLICATES REMOVED)
L18	338	S "CTXB"
L19	1	S L18 AND ALLERGY
L20	128	DUP REMOVE L18 (210 DUPLICATES REMOVED)

```
L21           0 S L20 AND ASTHMA
L22         193 S "ETXB"
L23           1 S L22 AND ALLERGY
L24          93 S L22 AND MUTANT
L25          24 DUP REMOVE L24 (69 DUPLICATES REMOVED)
L26      260989 S ASTHMA
L27       58201 S L26 AND TREATMENT
L28          22 S L27 AND CHOLERA TOXIN
L29          13 DUP REMOVE L28 (9 DUPLICATES REMOVED)

=> s l27 and heat labile toxin
L30           1 L27 AND HEAT LABILE TOXIN

=> d l30 cbib abs

L30  ANSWER 1 OF 1  CAPLUS  COPYRIGHT 2002 ACS
2002:272796   Document No. 136:293510  Anti-allergic vaccines comprising
     peptides of Fc portion of IgE .epsilon. heavy chain and carrier protein.
     Morsey, Mohamad Ali; Sheppard, Michael George; Wheeler, David Walter
     (Pfizer Products Inc., USA).  Eur. Pat. Appl. EP 1195161 A2 20020410, 38
     pp.  DESIGNATED STATES: R:  AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI,
     LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO.  (English).  CODEN: EPXXDW.
     APPLICATION: EP 2001-307247 20010824.  PRIORITY: US 2000-PV228989
     20000830.
AB   The present invention provides compns. and methods for the use of
     antigenic peptides derived from the Fc portion of the epsilon heavy chain
     of an IgE mol. as vaccines for the treatment and prevention of
     IgE-mediated allergic disorders.  In particular, the invention provides
     compns., methods for the treatment and prevention of
     IgE-mediated allergic disorders comprising an immunogenic amt. of one or
     more antigenic peptides derived from the CH3 domain or junction of
     Ch-3/CH4 domain of an IgE mol. and methods for the evaluation of IgE
     mediated allergies in dogs.  The allergic disorder is asthma,
     allergic rhinitis, gastrointestinal allergy, food allergy, eosinophilia,
     conjunctivitis, or glomerular nephritis.  The vaccine compns. may also
     comprises carrier protein such as KLH, PhoE, rmLT, TraT and gD from BhV-1
     virus; and adjuvant such as aluminum hydroxide, monophosphoryl lipid A,
     Thr-MDP, immunostimulatory oligonucleotide, cytokine, interleukin 12,
     interleukin 2, interleukin 1, saponin, cholera toxin, heat
     labile toxin, etc.


=> s (williams n?/au or hirst t?/au or bienenstock j?/au)
L31        7946 (WILLIAMS N?/AU OR HIRST T?/AU OR BIENENSTOCK J?/AU)

=> s l31 and Etx
L32          48 L31 AND ETX

=> dup remove l32
PROCESSING COMPLETED FOR L32
L33          16 DUP REMOVE L32 (32 DUPLICATES REMOVED)

=> d l33 1-16 cbib abs

L33  ANSWER 1 OF 16     MEDLINE                              DUPLICATE 1
2002150343  Document Number: 21877323.    PubMed ID: 11882700.   Contribution
     of the ADP-ribosylating and receptor-binding properties of cholera-like
     enterotoxins in modulating cytokine secretion by human intestinal
     epithelial cells. Soriani Marco; Bailey Lorna; Hirst Timothy R.
     (Department of Pathology and Microbiology, University of Bristol, Bristol
     BS8 1TD, UK. ) MICROBIOLOGY, (2002 Mar) 148 (Pt 3) 667-76.  Journal code:
     9430468. ISSN: 1350-0872. Pub. country: England: United Kingdom. Language:
     English.
```

AB When epithelial cells first encounter cholera toxin (Ctx) produced by Vibrio cholerae they secrete not only chloride ions responsible for causing diarrhoea, but also a number of cytokines that may contribute to the toxin's potent immunomodulatory properties. Much less is known about the ability of the heat-labile enterotoxin of Escherichia coli (**Etx**), a close homologue of Ctx, to elicit cytokine secretion by epithelial cells. This study shows that treatment of human intestinal epithelial T84 cells with **Etx** induces expression of IL-6, IL-10, IL-1R antagonist, as well as IL-1alpha and IL-1beta and low levels of IL-8. Such induction was totally dependent on the intrinsic ADP-ribosylating activity of the toxin A-subunit, and could be mimicked by cAMP-elevating agents, such as forskolin and dibutyryl cAMP. By comparison, neither an enzymically inactive mutant of **Etx** nor EtxB was able to induce cytokine secretion. The behaviour of Ctx and CtxB was very similar to that of **Etx** and EtxB, respectively. The spectrum of cytokines released by **Etx** and Ctx indicates that the toxins may create a local microenvironment that strongly biases the immune response towards an anti-inflammatory and a polarized Th2 response.

L33 ANSWER 2 OF 16 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
2001:521892 Document No.: PREV200100521892. Therapeutic agents and autoimmune diseases. **Williams, Neil Andrew (1); Hirst, Timothy Raymond;** Nashar, Toufic Osman. (1) 16 Old Coach Road, Cross, Axbridge, Somerset UK. Patent Info.: US 6287563 September 11, 2001. Official Gazette of the United States Patent and Trademark Office Patents, (Sep. 11, 2001) Vol. 1250, No. 2, pp. No Pagination. e-file. ISSN: 0098-1133. Language: English.

AB There is disclosed the use, as an agent in the treatment or the prevention of an autoimmune disease, of: (i) an agent having GM-1 binding activity, other than Ctx or **Etx**, or the B subunits of Ctx and **Etx** ; or (ii) an agent having an effect on GM-1 mediated intracellular signalling events, but no GM-1 binding activity. These agents may also be used in the treatment of human T cell leukaemia, in the prevention of transplant rejection or GVHD or in a vaccination method for vaccinating a mammalian subject.

L33 ANSWER 3 OF 16 CAPLUS COPYRIGHT 2002 ACS
2001:798749 Document No. 135:339267 Therapeutic agents. **Williams, Neil Andrew; Hirst, Timothy Raymond;** Nashar, Toufic Osman (UK). U.S. Pat. Appl. Publ. US 20010036917 A1 20011101, 53 pp., Cont.-in-part of U.S. 6,287,563. (English). CODEN: USXXCO. APPLICATION: US 2001-867914 20010530. PRIORITY: GB 1995-13733 19950705; US 1997-999458 19971229.

AB A method of treating diabetes in a mammalian subject by administering an agent capable of modulating a ganglioside GM-1 (GM-1) assocd. activity in an amt. effect to treat the disease; wherein agent is selected from the group consisting of cholera toxin (Ctx), enterotoxins (**Etx**), the B subunit of Ctx and the B subunit of **Etx**, mutants and derivs. thereof. along with co-administration of antigens which are not so linked to form a single active agent.

L33 ANSWER 4 OF 16 MEDLINE DUPLICATE 2
2001248162 Document Number: 21189275. PubMed ID: 11292779. Escherichia coli heat-labile enterotoxin B subunit is a more potent mucosal adjuvant than its vlosely related homologue, the B subunit of cholera toxin. Millar D G; **Hirst T R;** Snider D P. (Department of Pathology and Molecular Medicine, McMaster University, Hamilton, Ontario, Canada L8N 3Z5.. dmillar@uhnres.utoronto.ca) . INFECTION AND IMMUNITY, (2001 May) 69 (5) 3476-82. Journal code: 0246127. ISSN: 0019-9567. Pub. country: United States. Language: English.

AB Although cholera toxin (Ctx) and Escherichia coli heat-labile enterotoxin (**Etx**) are known to be potent mucosal adjuvants, it remains controversial whether the adjuvanticity of the holotoxins extends to their nontoxic, receptor-binding B subunits. Here, we have systematically

evaluated the comparative adjuvant properties of highly purified recombinant EtxB and CtxB. EtxB was found to be a more potent adjuvant than CtxB, stimulating responses to hen egg lysozyme when the two were coadministered to mice intranasally, as assessed by enhanced serum and secretory antibody titers as well as by stimulation of lymphocyte proliferation in spleen and draining lymph nodes. These results indicate that, although structurally very similar, EtxB and CtxB have strikingly different immunostimulatory properties and should not be considered equivalent as prospective vaccine adjuvants.

L33 ANSWER 5 OF 16 MEDLINE DUPLICATE 3

2001210820 Document Number: 21196418. PubMed ID: 11298654. Cholera toxin and Escherichia coli enterotoxin B-subunits inhibit macrophage-mediated antigen processing and presentation: evidence for antigen persistence in non-acidic recycling endosomal compartments. Millar D G; **Hirst T R** . (Department of Pathology and Microbiology, University of Bristol, School of Medical Sciences, Bristol BS8 1TD, UK.) CELLULAR MICROBIOLOGY, (2001 May) 3 (5) 311-29. Journal code: 100883691. ISSN: 1462-5814. Pub. country: England: United Kingdom. Language: English.

AB Cholera toxin (Ctx) and the closely related Escherichia coli heat-labile enterotoxin (**Etx**) not only act as mediators of diarrhoeal disease but also exert potent immunomodulatory properties on mammalian immune systems. The toxins normally exert their diarrhoeagenic effects by initiating receptor-mediated uptake into vesicles that enter a retrograde trafficking pathway, circumventing degradative compartments and targeting them to the trans-Golgi network (TGN) and endoplasmic reticulum. Here, we examine whether receptor-mediated binding and cellular entry by the toxin B-subunits also lead to concomitant changes in uptake and trafficking of exogenous antigens that could contribute to the potent immunomodulatory properties of these toxins. Treatment of the macrophage (J774.2) cell line with **Etx** B-subunit (EtxB) resulted in EtxB transport to the TGN and also led to the formation of large, translucent, non-acidic, EtxB-devoid vacuoles. When exogenous antigens were added, EtxB-treated cells were found to be proficient in both internalization of ovalbumin (OVA) and phagocytosis of bacterial particles. However, the internalized OVA, instead of trafficking along a lysosome-directed endocytic pathway via acidified endosomes, persisted in a non-acidic, light-density compartment that was distinct from the translucent vacuoles. The rerouted OVA did not co-localize with the endosomal markers rab5 or rab11, nor with EtxB, but was retained in a transferrin receptor-positive compartment. The failure of OVA to enter the late endosomal/lysosomal compartments correlated with a striking inhibition of OVA peptide processing and presentation to OVA-responsive CD4+ T-cells. CtxB also modulated OVA trafficking and inhibited antigen presentation. These findings demonstrate that the B-subunits of Ctx and **Etx** alter the progression of exogenous antigens along the endocytic processing pathway, and prevent or delay efficient epitope presentation and T-cell stimulation. The formation of such 'antigen depots' could contribute to the immunomodulatory properties of these bacterial virulence determinants.

L33 ANSWER 6 OF 16 MEDLINE DUPLICATE 4

2001221761 Document Number: 20562508. PubMed ID: 11111925. Immune modulation by the cholera-like enterotoxin B-subunits: from adjuvant to immunotherapeutic. **Williams N A.** (University of Bristol, Department of Pathology and Microbiology, School of Medical Sciences, UK.. Neil.a.williams@bris.ac.uk) . INTERNATIONAL JOURNAL OF MEDICAL MICROBIOLOGY, (2000 Oct) 290 (4-5) 447-53. Ref: 43. Journal code: 100898849. ISSN: 1438-4221. Pub. country: GERMANY: Germany, Federal Republic of. Language: English.

AB Cholera toxin (Ctx) and its close relative, Escherichia coli heat-labile enterotoxin (**Etx**) have long been established as potent mucosal and systemic adjuvants. Problems arising from their inherent toxicity have, however, precluded human use. Here we describe findings which

demonstrate that contrary to the established dogma the non-toxic B-subunit of **Etx** (EtxB) is a highly potent mucosal adjuvant capable of potentiating protective immunity to viral infection. The mechanisms which underlie this activity arise from an ability to trigger specific signaling processes in lymphocyte populations which modulate differentially their activation, differentiation and survival. The elucidation of these properties has led to the further use of EtxB as an agent capable of preventing the establishment of autoimmune diseases. The basis for these activities and their potential applicability to human therapies are discussed.

L33 ANSWER 7 OF 16 MEDLINE DUPLICATE 5
2000445484 Document Number: 20450026. PubMed ID: 10994530. Cholera toxin and related enterotoxins: a cell biological and immunological perspective. de Haan L; **Hirst T R**. (Department of Pathology and Microbiology, School of Medical Sciences, University of Bristol, United Kingdom.) JOURNAL OF NATURAL TOXINS, (2000 Aug) 9 (3) 281-97. Ref: 126. Journal code: 9208016. ISSN: 1058-8108. Pub. country: United States. Language: English.

AB Cholera toxin (Ctx) from Vibrio cholerae and the closely related Escherichia coli heat-labile enterotoxin (**Etx**) are the primary virulence factors responsible for causing cholera and traveller's diarrhea, respectively. Studies on the mode of action of these toxins on gut epithelial cells have revealed important insights into the mechanisms of toxin uptake and trafficking in eukaryotic cells. However, of perhaps even greater fascination have been the discoveries that Ctx and **Etx** exhibit remarkable immunological properties. When either of these toxins is administered via mucosal routes, it triggers a potent mucosal and systemic anti-toxin immune response. By contrast, local or systemic immunization with other soluble protein antigens usually stimulates only a meagre immune response, or results in a state of immunological tolerance. Even more striking are the findings that when Ctx or **Etx** are mixed with heterologous antigens, they function as adjuvants, leading to stimulation of mucosal responses to the admixed antigen, and the abrogation of oral tolerance. In addition, recent observations have shown that the receptor-binding component of these toxins can down-regulate inflammatory diseases associated with the induction of autoimmune disorders such as rheumatoid arthritis, diabetes, and multiple sclerosis. While the underlying mechanisms responsible for these remarkable properties have yet to be resolved, it is clear that the toxins' ability to bind to cell surface receptors plays an important role in their potent immunogenicity, adjuvanticity, and immunotherapeutic properties. This review provides an overview of the latest developments within the Ctx/**Etx** field, with a special emphasis on the cell entry mechanisms and immunomodulatory action of Ctx/**Etx** and their component subunits.

L33 ANSWER 8 OF 16 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
2001:183956 Document No.: PREV200100183956. Does the B-subunit of Shiga toxin have immunomodulatory properties. Kenny, M. J. (1); Fraser, S. A. (1); Pitman, R. S. (1); **Williams, N. A. (1); Hirst, T. R. (1)** . (1) Department of Pathology and Microbiology, University of Bristol, University Walk, Bristol, BS8 1TD UK. IJMM International Journal of Medical Microbiology, (October, 2000) Vol. 290, No. 4-5, Supplement 30, pp. A85. print. Meeting Info.: 9th European Workshop on Bacterial Protein Toxins Saint Maxime, France June 27-July 02, 1999 ISSN: 1438-4221. Language: English. Summary Language: English.

L33 ANSWER 9 OF 16 MEDLINE DUPLICATE 6
1999134317 Document Number: 99134317. PubMed ID: 9933586. Structural basis for the differential toxicity of cholera toxin and Escherichia coli heat-labile enterotoxin. Construction of hybrid toxins identifies the A2-domain as the determinant of differential toxicity. Rodighiero C; Aman

A T; Kenny M J; Moss J; Lencer W I; **Hirst T R.** (Department of Pathology and Microbiology, University of Bristol, School of Medical Sciences, Bristol BS8 1TD, United Kingdom.) JOURNAL OF BIOLOGICAL CHEMISTRY, (1999 Feb 12) 274 (7) 3962-9. Journal code: 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.

AB Cholera toxin (Ctx) and E. coli heat-labile enterotoxin (**Etx**) are structurally and functionally similar AB5 toxins with over 80% sequence identity. When their action in polarized human epithelial (T84) cells was monitored by measuring toxin-induced Cl- ion secretion, Ctx was found to be the more potent of the two toxins. Here, we examine the structural basis for this difference in toxicity by engineering a set of mutant and hybrid toxins and testing their activity in T84 cells. This revealed that the differential toxicity of Ctx and **Etx** was (i) not due to differences in the A-subunit's C-terminal KDEL targeting motif (which is RDEL in **Etx**), as a KDEL to RDEL substitution had no effect on cholera toxin activity; (ii) not attributable to the enzymatically active A1-fragment, as hybrid toxins in which the A1-fragment in Ctx was substituted for that of **Etx** (and vice versa) did not alter relative toxicity; and (iii) not due to the B-subunit, as the replacement of the B-subunit in Ctx for that of **Etx** caused no alteration in toxicity, thus excluding the possibility that the broader receptor specificity of EtxB is responsible for reduced activity. Remarkably, the difference in toxicity could be mapped to a 10-amino acid segment of the A2-fragment that penetrates the central pore of the B-subunit pentamer. A comparison of the in vitro stability of two hybrid toxins, differing only in this 10-amino acid segment, revealed that the Ctx A2-segment conferred a greater stability to the interaction between the A- and B-subunits than the corresponding segment from **Etx** A2. This suggests that the reason for the relative potency of Ctx compared with **Etx** stems from the increased ability of the A2-fragment of Ctx to maintain holotoxin stability during uptake and transport into intestinal epithelia.

L33 ANSWER 10 OF 16 CAPLUS COPYRIGHT 2002 ACS

2000:883742 Document No. 135:44842 Immune modulation by the cholera-like enterotoxin B-subunits: From adjuvant to immunotherapeutic. Pitman, Richard S.; **Hirst, Timothy R.; Williams, Neil A.** (Division of Gastroenterology, Department of Medicine, Brigham and Women's Hospital, Boston, MA, 02115, USA). Recent Research Developments in Immunology, 1(Pt. 2), 497-511 (English) 1999. CODEN: RRDIB8. Publisher: Research Signpost.

AB A review with 59 refs. Cholera toxin (Ctx) and its close relative, Escherichia coli heat-labile enterotoxin (**Etx**) have long been established as potent mucosal and systemic adjuvants. Problems arising from their inherent toxicity have, however, precluded human use. Here the authors describe findings which demonstrate that the non-toxic B-subunit of **Etx** (EtxB) is a highly potent mucosal adjuvant capable of potentiating protective immunity to viral infection. The mechanisms which underlie this activity arise from an ability to trigger specific signaling processes in lymphocyte populations which modulate differentially their activation, differentiation, and survival. The elucidation of these properties has led to the further use of EtxB as an agent capable of preventing the establishment of autoimmune diseases. The basis for these activities and their potential applicability to human therapies are discussed.

L33 ANSWER 11 OF 16 CAPLUS COPYRIGHT 2002 ACS

1999:36136 Document No. 130:219334 Differential activity of cholera toxin and E. coli enterotoxin: construction and purification of mutant and hybrid derivatives. Rodighiero, C.; Aman, A. T.; Lencer, W. I.; **Hirst, T. R.** (Department of Pathology and Microbiology, School of Medical Sciences, University of Bristol, Bristol, BS8 1TD, UK). Biochemical Society Transactions, 26(4), S364 (English) 1998. CODEN:

BCSTB5. ISSN: 0300-5127. Publisher: Portland Press Ltd..

AB To det. whether the differential toxicity of cholera toxin (Ctx) and Escherichia enterotoxin (**Etx**) lies within the A- or B- subunits of the mols., chimeras have been engineered which comprise portions of the A-subunit of Ctx complexed with the B-subunit of **Etx** and vice versa. A mutant cholera toxin in which the C-terminal ER retention signal (KDEL) was substituted for RDEL found in **Etx**, was also prepd. Here the authors describe the genetic construction of mutant and hybrid toxins and a method for their purifn.

L33 ANSWER 12 OF 16 CAPLUS COPYRIGHT 2002 ACS

1999:36021 Document No. 130:164241 Receptor mediated apoptosis of CD8+T cells by the B subunits of cholera-like enterotoxins. Pitman, Richard S.; **Hirst, Timothy R.**; Nashar, Toufic O.; **Williams, Neil A.** (Department of Pathology and Microbilogy, School of Medical Sciences, University of Bristol, Bristol, BS8 1TD, UK). Biochemical Society Transactions, 26(4), S338 (English) 1998. CODEN: BCSTB5. ISSN: 0300-5127. Publisher: Portland Press Ltd..

AB Heat-labile enterotoxin (**Etx**) B subunit (EtxB) and cholera toxin (Ctx) B subunit directly mediate apoptosis of CD8+T cells through an interaction with GM1, present on lymphocyte cell surfaces. Although the precise signaling pathways which mediate EtxB induced cellular activation and apoptosis remain unknown, it has been demonstrated that resp. levels of ceramide and MAPK (mitogen-activated protein kinase) activity remain unaltered in both T and B lymphocytes upon addn. of EtxB, thereby excluding a role for these signaling mechanisms.

L33 ANSWER 13 OF 16 CAPLUS COPYRIGHT 2002 ACS

1997:181160 Document No. 126:170385 Therapeutic agents and autoimmune diseases. **Williams, Neil Andrew; Hirst, Timothy Raymond**; Nashar, Toufic Osman (University of Bristol, UK; Williams, Neil, Andrew; Hirst, Timothy, Raymond; Nashar, Toufic, Osman). PCT Int. Appl. WO 9702045 A1 19970123, 62 pp. DESIGNATED STATES: W: AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, NL, PT, SE. (English). CODEN: PIXXD2. APPLICATION: WO 1996-GB1614 19960705. PRIORITY: GB 1995-13733 19950705.

AB There is disclosed the use, as an agent in the treatment or the prevention of an autoimmune disease, of: (i) an agent having GM-1 binding activity, other than Ctx or **Etx**, or the B subunits of Ctx and **Etx**; or (ii) an agent having an effect on GM-1 mediated intracellular signalling events, but no GM-1 binding activity. These agents may also be used in the treatment of human T cell leukemia, in the prevention of transplant rejection or GVHD or in a vaccination method for vaccinating a mammalian subject.

L33 ANSWER 14 OF 16 MEDLINE DUPLICATE 7

96324796 Document Number: 96324796. PubMed ID: 8671661. Cross-linking of cell surface ganglioside GM1 induces the selective apoptosis of mature CD8+ T lymphocytes. Nahar T O; **Williams N A; Hirst T R**. (Research School of Biosciences, University of Kent, Canterbury, UK.) INTERNATIONAL IMMUNOLOGY, (1996 May) 8 (5) 731-6. Ref: 24. Journal code: 8916182. ISSN: 0953-8178. Pub. country: ENGLAND: United Kingdom. Language: English.

AB Gangliosides are glycosphingolipids found ubiquitously on the surface of mammalian cells. They contain a ceramide tail that is inserted into the membrane and exposed carbohydrate and sialic acid moieties. The non-toxic B subunit oligomer (EtxB) of Escherichia coli heat-labile enterotoxin (**Etx**) is a potent immunogen in vivo and has profound modulatory effects on EtxB-primed lymphocytes in vitro, properties which are dependent on its ability to bind to GM1 ganglioside receptors. Here, it is

shown that cross-linking GM1 by EtxB causes a differential effect on mature CD4(+) and CD8(+) T cells from lymph node cultures proliferating in response to an unrelated antigen, ovalbumin. Addition of EtxB to such cultures led to the complete depletion of CD8(+) T cells compared with enhanced activation of CD4(+) cells [as measured by expression of CD25 (IL-2Ralpha)]. By contrast, addition of a mutant EtxB, EtxB(G33D), which does not bind to GM1, failed to trigger CD8(+) T cell depletion. When EtxB was added to isolated non-immune CD8(+) lymphocytes rapid (12-18 h) alterations in nuclear morphology and the appearance of sub-G0/G1 levels of DNA were induced; properties which are characteristic of cells undergoing apoptosis. EtxB(G33D) failed to trigger apoptosis, indicating that the induction of the apoptotic signal was dependent on the binding of GM1. These findings provide an insight into the potent immunogenicity and immunomodulatory properties of E. coli enterotoxins as well as heralding a novel method for the selective induction of apoptosis in mature CD8(+) T lymphocytes.

L33 ANSWER 15 OF 16 MEDLINE DUPLICATE 8

94331983 Document Number: 94331983. PubMed ID: 8054855. Purification of the B-subunit oligomer of Escherichia coli heat-labile enterotoxin by heterologous expression and secretion in a marine vibrio. Amin T; **Hirst T R.** (Biological Laboratory, The University, Canterbury, Kent, United Kingdom.) PROTEIN EXPRESSION AND PURIFICATION, (1994 Apr) 5 (2) 198-204. Journal code: 9101496. ISSN: 1046-5928. Pub. country: United States. Language: English.

AB Heat-labile enterotoxins (**Etx**) are plasmid-encoded, multimeric proteins produced by certain diarrheagenic strains of Escherichia coli. The nontoxic, receptor-binding B subunit (EtxB) of such toxins may be useful as a component of vaccines against enterotoxigenic E. coli, or as a carrier for the delivery of heterologous epitopes to the mucosal immune system. Here we describe a simple method for the purification of EtxB from a marine vibrio harboring a broad-host range controlled expression vector containing the etxB gene. Induction of EtxB resulted in its specific secretion to the medium, to a concentration of greater than 25 mg/liter of culture. The techniques of ultrafiltration and hydrophobic interaction chromatography were used to purify EtxB to homogeneity from the medium of this organism (with a yield of 60.7%). EtxB-epitope fusion proteins were also successfully expressed and secreted in this marine vibrio, suggesting that this system may be of general use in the preparation of EtxB-based vaccines.

L33 ANSWER 16 OF 16 MEDLINE DUPLICATE 9

92374846 Document Number: 92374846. PubMed ID: 1324389. A homologue of the Escherichia coli DsbA protein involved in disulphide bond formation is required for enterotoxin biogenesis in Vibrio cholerae. Yu J; Webb H; **Hirst T R.** (Biological Laboratory, University of Kent, Canterbury, UK.) MOLECULAR MICROBIOLOGY, (1992 Jul) 6 (14) 1949-58. Journal code: 8712028. ISSN: 0950-382X. Pub. country: ENGLAND: United Kingdom. Language: English.

AB A strain of Vibrio cholerae, which had been engineered to express high levels of the non-toxic B subunit (EtxB) of Escherichia coli heat-labile enterotoxin, was subjected to transposon (TnphoA) mutagenesis. Two chromosomal TnphoA insertion mutations of the strain were isolated that showed a severe defect in the amount of EtxB produced. The loci disrupted by TnphoA in the two mutant derivatives were cloned and sequenced, and this revealed that the transposon had inserted at different sites in the same gene. The open reading frame of the gene predicts a 200-amino-acid exported protein, with a Cys-X-X-Cys motif characteristic of thioredoxin, protein disulphide isomerase, and DsbA (a periplasmic protein required for disulphide bond formation in E. coli). The V. cholerae protein exhibited 40% identity with the DsbA protein of E. coli, including 90% identity in the region of the active-site motif. Introduction of a plasmid encoding E. coli DsbA into the V. cholerae TnphoA derivatives was found to restore

enterotoxin formation, whilst expression of **Etx** or EtxB in a dsbA mutant of E. coli confirmed that DsbA is required for enterotoxin formation in E. coli. These results suggest that, since each EtxB subunit contains a single intramolecular disulphide bond, a transient intermolecular interaction with DsbA occurs during toxin subunit folding which catalyses formation of the disulphide in vivo.

=> d his

(FILE 'HOME' ENTERED AT 09:31:40 ON 01 JUL 2002)

FILE 'MEDLINE, EMBASE, BIOSIS, SCISEARCH, CAPLUS' ENTERED AT 09:32:05 ON 01 JUL 2002

```
L1         51040 S GANGLIOSIDE
L2          2708 S L1 AND MODULAT?
L3           219 S L2 AND AGENT
L4           105 S L3 AND TREATMENT
L5             1 S L4 AND ALLERGY
L6             6 S L4 AND HYPERSENSITIVITY
L7             2 DUP REMOVE L6 (4 DUPLICATES REMOVED)
L8           766 S "ETX"
L9             8 S L8 AND GANGLIOSIDE
L10            4 DUP REMOVE L9 (4 DUPLICATES REMOVED)
L11         9952 S "CTX"
L12           64 S L11 AND GANGLIOSIDE
L13            0 S L12 AND HYPERSENSITIVITY
L14            0 S L12 AND IGE
L15            0 S L12 AND ALLERGY
L16            1 S L12 AND MUTANT
L17           20 DUP REMOVE L12 (44 DUPLICATES REMOVED)
L18          338 S "CTXB"
L19            1 S L18 AND ALLERGY
L20          128 DUP REMOVE L18 (210 DUPLICATES REMOVED)
L21            0 S L20 AND ASTHMA
L22          193 S "ETXB"
L23            1 S L22 AND ALLERGY
L24           93 S L22 AND MUTANT
L25           24 DUP REMOVE L24 (69 DUPLICATES REMOVED)
L26       260989 S ASTHMA
L27        58201 S L26 AND TREATMENT
L28           22 S L27 AND CHOLERA TOXIN
L29           13 DUP REMOVE L28 (9 DUPLICATES REMOVED)
L30            1 S L27 AND HEAT LABILE TOXIN
L31         7946 S (WILLIAMS N?/AU OR HIRST T?/AU OR BIENENSTOCK J?/AU)
L32           48 S L31 AND ETX
L33           16 DUP REMOVE L32 (32 DUPLICATES REMOVED)

=> s l31 and Ctx
L34           38 L31 AND CTX

=> dup remove l34
PROCESSING COMPLETED FOR L34
L35           15 DUP REMOVE L34 (23 DUPLICATES REMOVED)

=> d l35 1-15 cbib abs

L35  ANSWER 1 OF 15     MEDLINE                                  DUPLICATE 1
2002150343  Document Number: 21877323.    PubMed ID: 11882700.   Contribution
     of the ADP-ribosylating and receptor-binding properties of cholera-like
     enterotoxins in modulating cytokine secretion by human intestinal
     epithelial cells. Soriani Marco; Bailey Lorna; Hirst Timothy R.
     (Department of Pathology and Microbiology, University of Bristol, Bristol
```

BS8 1TD, UK.) MICROBIOLOGY, (2002 Mar) 148 (Pt 3) 667-76. Journal code: 9430468. ISSN: 1350-0872. Pub. country: England: United Kingdom. Language: English.

AB When epithelial cells first encounter cholera toxin (**Ctx**) produced by Vibrio cholerae they secrete not only chloride ions responsible for causing diarrhoea, but also a number of cytokines that may contribute to the toxin's potent immunomodulatory properties. Much less is known about the ability of the heat-labile enterotoxin of Escherichia coli (Etx), a close homologue of **Ctx**, to elicit cytokine secretion by epithelial cells. This study shows that treatment of human intestinal epithelial T84 cells with Etx induces expression of IL-6, IL-10, IL-1R antagonist, as well as IL-1alpha and IL-1beta and low levels of IL-8. Such induction was totally dependent on the intrinsic ADP-ribosylating activity of the toxin A-subunit, and could be mimicked by cAMP-elevating agents, such as forskolin and dibutyryl cAMP. By comparison, neither an enzymically inactive mutant of Etx nor EtxB was able to induce cytokine secretion. The behaviour of **Ctx** and CtxB was very similar to that of Etx and EtxB, respectively. The spectrum of cytokines released by Etx and **Ctx** indicates that the toxins may create a local microenvironment that strongly biases the immune response towards an anti-inflammatory and a polarized Th2 response.

L35 ANSWER 2 OF 15 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.

2001:521892 Document No.: PREV200100521892. Therapeutic agents and autoimmune diseases. **Williams, Neil Andrew (1); Hirst, Timothy Raymond;** Nashar, Toufic Osman. (1) 16 Old Coach Road, Cross, Axbridge, Somerset UK. Patent Info.: US 6287563 September 11, 2001. Official Gazette of the United States Patent and Trademark Office Patents, (Sep. 11, 2001) Vol. 1250, No. 2, pp. No Pagination. e-file. ISSN: 0098-1133. Language: English.

AB There is disclosed the use, as an agent in the treatment or the prevention of an autoimmune disease, of: (i) an agent having GM-1 binding activity, other than **Ctx** or Etx, or the B subunits of **Ctx** and Etx; or (ii) an agent having an effect on GM-1 mediated intracellular signalling events, but no GM-1 binding activity. These agents may also be used in the treatment of human T cell leukaemia, in the prevention of transplant rejection or GVHD or in a vaccination method for vaccinating a mammalian subject.

L35 ANSWER 3 OF 15 CAPLUS COPYRIGHT 2002 ACS

2001:798749 Document No. 135:339267 Therapeutic agents. **Williams, Neil Andrew; Hirst, Timothy Raymond;** Nashar, Toufic Osman (UK). U.S. Pat. Appl. Publ. US 20010036917 A1 20011101, 53 pp., Cont.-in-part of U.S. 6,287,563. (English). CODEN: USXXCO. APPLICATION: US 2001-867914 20010530. PRIORITY: GB 1995-13733 19950705; US 1997-999458 19971229.

AB A method of treating diabetes in a mammalian subject by administering an agent capable of modulating a ganglioside GM-1 (GM-1) assocd. activity in an amt. effect to treat the disease; wherein agent is selected from the group consisting of cholera toxin (**Ctx**), enterotoxins (Etx), the B subunit of **Ctx** and the B subunit of Etx, mutants and derivs. thereof. along with co-administration of antigens which are not so linked to form a single active agent.

L35 ANSWER 4 OF 15 MEDLINE DUPLICATE 2

2001248162 Document Number: 21189275. PubMed ID: 11292779. Escherichia coli heat-labile enterotoxin B subunit is a more potent mucosal adjuvant than its vlosely related homologue, the B subunit of cholera toxin. Millar D G; **Hirst T R;** Snider D P. (Department of Pathology and Molecular Medicine, McMaster University, Hamilton, Ontario, Canada L8N 3Z5.. dmillar@uhnres.utoronto.ca) . INFECTION AND IMMUNITY, (2001 May) 69 (5) 3476-82. Journal code: 0246127. ISSN: 0019-9567. Pub. country: United States. Language: English.

AB Although cholera toxin (**Ctx**) and Escherichia coli heat-labile

enterotoxin (Etx) are known to be potent mucosal adjuvants, it remains controversial whether the adjuvanticity of the holotoxins extends to their nontoxic, receptor-binding B subunits. Here, we have systematically evaluated the comparative adjuvant properties of highly purified recombinant EtxB and CtxB. EtxB was found to be a more potent adjuvant than CtxB, stimulating responses to hen egg lysozyme when the two were coadministered to mice intranasally, as assessed by enhanced serum and secretory antibody titers as well as by stimulation of lymphocyte proliferation in spleen and draining lymph nodes. These results indicate that, although structurally very similar, EtxB and CtxB have strikingly different immunostimulatory properties and should not be considered equivalent as prospective vaccine adjuvants.

L35 ANSWER 5 OF 15 MEDLINE DUPLICATE 3

2001192726 Document Number: 21105201. PubMed ID: 11160664. Protective mucosal immunity to ocular herpes simplex virus type 1 infection in mice by using Escherichia coli heat-labile enterotoxin B subunit as an adjuvant. Richards C M; Aman A T; **Hirst T R**; Hill T J; **Williams N A**. (Department of Pathology and Microbiology, School of Medical Sciences, University of Bristol, Bristol BS8 1TD, United Kingdom.. Claire.M.Richards@bristol.ac.uk) . JOURNAL OF VIROLOGY, (2001 Feb) 75 (4) 1664-71. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB The potential of nontoxic recombinant B subunits of cholera toxin (rCtxB) and its close relative Escherichia coli heat-labile enterotoxin (rEtxB) to act as mucosal adjuvants for intranasal immunization with herpes simplex virus type 1 (HSV-1) glycoproteins was assessed. Doses of 10 microg of rEtxB or above with 10 microg of HSV-1 glycoproteins elicited high serum and mucosal anti-HSV-1 titers comparable with that obtained using CtxB (10 microg) with a trace (0.5 microg) of whole toxin (**Ctx**-CtxB). By contrast, doses of rCtxB up to 100 microg elicited only meager anti-HSV-1 responses. As for **Ctx**-CtxB, rEtxB resulted in a Th2-biased immune response with high immunoglobulin G1 (IgG1)/IgG2a antibody ratios and production of interleukin 4 (IL-4) and IL-10 as well as gamma interferon by proliferating T cells. The protective efficacy of the immune response induced using rEtxB as an adjuvant was assessed following ocular challenge of immunized and mock-immunized mice. Epithelial disease was observed in both groups, but the immunized mice recovered by day 6 whereas mock-immunized mice developed more severe corneal disease leading to stromal keratitis. In addition, a significant reduction in the incidence of lid disease and zosteriform spread was observed in immunized animals and there was no encephalitis compared with 95% encephalitis in mock-immunized mice. The potential of such mucosal adjuvants for use in human vaccines against pathogens such as HSV-1 is discussed.

L35 ANSWER 6 OF 15 MEDLINE DUPLICATE 4

2001210820 Document Number: 21196418. PubMed ID: 11298654. Cholera toxin and Escherichia coli enterotoxin B-subunits inhibit macrophage-mediated antigen processing and presentation: evidence for antigen persistence in non-acidic recycling endosomal compartments. Millar D G; **Hirst T R** . (Department of Pathology and Microbiology, University of Bristol, School of Medical Sciences, Bristol BS8 1TD, UK.) CELLULAR MICROBIOLOGY, (2001 May) 3 (5) 311-29. Journal code: 100883691. ISSN: 1462-5814. Pub. country: England: United Kingdom. Language: English.

AB Cholera toxin (**Ctx**) and the closely related Escherichia coli heat-labile enterotoxin (Etx) not only act as mediators of diarrhoeal disease but also exert potent immunomodulatory properties on mammalian immune systems. The toxins normally exert their diarrhoeagenic effects by initiating receptor-mediated uptake into vesicles that enter a retrograde trafficking pathway, circumventing degradative compartments and targeting them to the trans-Golgi network (TGN) and endoplasmic reticulum. Here, we examine whether receptor-mediated binding and cellular entry by the toxin B-subunits also lead to concomitant changes in uptake and trafficking of

exogenous antigens that could contribute to the potent immunomodulatory properties of these toxins. Treatment of the macrophage (J774.2) cell line with Etx B-subunit (EtxB) resulted in EtxB transport to the TGN and also led to the formation of large, translucent, non-acidic, EtxB-devoid vacuoles. When exogenous antigens were added, EtxB-treated cells were found to be proficient in both internalization of ovalbumin (OVA) and phagocytosis of bacterial particles. However, the internalized OVA, instead of trafficking along a lysosome-directed endocytic pathway via acidified endosomes, persisted in a non-acidic, light-density compartment that was distinct from the translucent vacuoles. The rerouted OVA did not co-localize with the endosomal markers rab5 or rab11, nor with EtxB, but was retained in a transferrin receptor-positive compartment. The failure of OVA to enter the late endosomal/lysosomal compartments correlated with a striking inhibition of OVA peptide processing and presentation to OVA-responsive CD4+ T-cells. CtxB also modulated OVA trafficking and inhibited antigen presentation. These findings demonstrate that the B-subunits of **Ctx** and Etx alter the progression of exogenous antigens along the endocytic processing pathway, and prevent or delay efficient epitope presentation and T-cell stimulation. The formation of such 'antigen depots' could contribute to the immunomodulatory properties of these bacterial virulence determinants.

L35 ANSWER 7 OF 15 MEDLINE DUPLICATE 5

2001221761 Document Number: 20562508. PubMed ID: 11111925. Immune modulation by the cholera-like enterotoxin B-subunits: from adjuvant to immunotherapeutic. **Williams N A**. (University of Bristol, Department of Pathology and Microbiology, School of Medical Sciences, UK.. Neil.a.williams@bris.ac.uk) . INTERNATIONAL JOURNAL OF MEDICAL MICROBIOLOGY, (2000 Oct) 290 (4-5) 447-53. Ref: 43. Journal code: 100898849. ISSN: 1438-4221. Pub. country: GERMANY: Germany, Federal Republic of. Language: English.

AB Cholera toxin (**Ctx**) and its close relative, Escherichia coli heat-labile enterotoxin (Etx) have long been established as potent mucosal and systemic adjuvants. Problems arising from their inherent toxicity have, however, precluded human use. Here we describe findings which demonstrate that contrary to the established dogma the non-toxic B-subunit of Etx (EtxB) is a highly potent mucosal adjuvant capable of potentiating protective immunity to viral infection. The mechanisms which underlie this activity arise from an ability to trigger specific signaling processes in lymphocyte populations which modulate differentially their activation, differentiation and survival. The elucidation of these properties has led to the further use of EtxB as an agent capable of preventing the establishment of autoimmune diseases. The basis for these activities and their potential applicability to human therapies are discussed.

L35 ANSWER 8 OF 15 MEDLINE DUPLICATE 6

2000445484 Document Number: 20450026. PubMed ID: 10994530. Cholera toxin and related enterotoxins: a cell biological and immunological perspective. de Haan L; **Hirst T R**. (Department of Pathology and Microbiology, School of Medical Sciences, University of Bristol, United Kingdom.) JOURNAL OF NATURAL TOXINS, (2000 Aug) 9 (3) 281-97. Ref: 126. Journal code: 9208016. ISSN: 1058-8108. Pub. country: United States. Language: English.

AB Cholera toxin (**Ctx**) from Vibrio cholerae and the closely related Escherichia coli heat-labile enterotoxin (Etx) are the primary virulence factors responsible for causing cholera and traveller's diarrhea, respectively. Studies on the mode of action of these toxins on gut epithelial cells have revealed important insights into the mechanisms of toxin uptake and trafficking in eukaryotic cells. However, of perhaps even greater fascination have been the discoveries that **Ctx** and Etx exhibit remarkable immunological properties. When either of these toxins is administered via mucosal routes, it triggers a potent mucosal and systemic anti-toxin immune response. By contrast, local or systemic

immunization with other soluble protein antigens usually stimulates only a meagre immune response, or results in a state of immunological tolerance. Even more striking are the findings that when **Ctx** or Etx are mixed with heterologous antigens, they function as adjuvants, leading to stimulation of mucosal responses to the admixed antigen, and the abrogation of oral tolerance. In addition, recent observations have shown that the receptor-binding component of these toxins can down-regulate inflammatory diseases associated with the induction of autoimmune disorders such as rheumatoid arthritis, diabetes, and multiple sclerosis. While the underlying mechanisms responsible for these remarkable properties have yet to be resolved, it is clear that the toxins' ability to bind to cell surface receptors plays an important role in their potent immunogenicity, adjuvanticity, and immunotherapeutic properties. This review provides an overview of the latest developments within the **Ctx**/Etx field, with a special emphasis on the cell entry mechanisms and immunomodulatory action of **Ctx**/Etx and their component subunits.

L35 ANSWER 9 OF 15 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
2001:183956 Document No.: PREV200100183956. Does the B-subunit of Shiga toxin have immunomodulatory properties. Kenny, M. J. (1); Fraser, S. A. (1); Pitman, R. S. (1); **Williams, N. A. (1); Hirst, T. R. (1)** . (1) Department of Pathology and Microbiology, University of Bristol, University Walk, Bristol, BS8 1TD UK. IJMM International Journal of Medical Microbiology, (October, 2000) Vol. 290, No. 4-5, Supplement 30, pp. A85. print. Meeting Info.: 9th European Workshop on Bacterial Protein Toxins Saint Maxime, France June 27-July 02, 1999 ISSN: 1438-4221. Language: English. Summary Language: English.

L35 ANSWER 10 OF 15 MEDLINE DUPLICATE 7
1999134317 Document Number: 99134317. PubMed ID: 9933586. Structural basis for the differential toxicity of cholera toxin and Escherichia coli heat-labile enterotoxin. Construction of hybrid toxins identifies the A2-domain as the determinant of differential toxicity. Rodighiero C; Aman A T; Kenny M J; Moss J; Lencer W I; **Hirst T R**. (Department of Pathology and Microbiology, University of Bristol, School of Medical Sciences, Bristol BS8 1TD, United Kingdom.) JOURNAL OF BIOLOGICAL CHEMISTRY, (1999 Feb 12) 274 (7) 3962-9. Journal code: 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.
AB Cholera toxin (**Ctx**) and E. coli heat-labile enterotoxin (Etx) are structurally and functionally similar AB5 toxins with over 80% sequence identity. When their action in polarized human epithelial (T84) cells was monitored by measuring toxin-induced Cl- ion secretion, **Ctx** was found to be the more potent of the two toxins. Here, we examine the structural basis for this difference in toxicity by engineering a set of mutant and hybrid toxins and testing their activity in T84 cells. This revealed that the differential toxicity of **Ctx** and Etx was (i) not due to differences in the A-subunit's C-terminal KDEL targeting motif (which is RDEL in Etx), as a KDEL to RDEL substitution had no effect on cholera toxin activity; (ii) not attributable to the enzymatically active A1-fragment, as hybrid toxins in which the A1-fragment in **Ctx** was substituted for that of Etx (and vice versa) did not alter relative toxicity; and (iii) not due to the B-subunit, as the replacement of the B-subunit in **Ctx** for that of Etx caused no alteration in toxicity, thus excluding the possibility that the broader receptor specificity of EtxB is responsible for reduced activity. Remarkably, the difference in toxicity could be mapped to a 10-amino acid segment of the A2-fragment that penetrates the central pore of the B-subunit pentamer. A comparison of the in vitro stability of two hybrid toxins, differing only in this 10-amino acid segment, revealed that the **Ctx** A2-segment conferred a greater stability to the interaction between the A- and B-subunits than the corresponding segment from Etx A2. This suggests that the reason for the relative potency of

Ctx compared with Etx stems from the increased ability of the A2-fragment of **Ctx** to maintain holotoxin stability during uptake and transport into intestinal epithelia.

L35 ANSWER 11 OF 15 CAPLUS COPYRIGHT 2002 ACS
2000:883742 Document No. 135:44842 Immune modulation by the cholera-like enterotoxin B-subunits: From adjuvant to immunotherapeutic. Pitman, Richard S.; **Hirst, Timothy R.; Williams, Neil A.** (Division of Gastroenterology, Department of Medicine, Brigham and Women's Hospital, Boston, MA, 02115, USA). Recent Research Developments in Immunology, 1(Pt. 2), 497-511 (English) 1999. CODEN: RRDIB8. Publisher: Research Signpost.

AB A review with 59 refs. Cholera toxin (**Ctx**) and its close relative, Escherichia coli heat-labile enterotoxin (Etx) have long been established as potent mucosal and systemic adjuvants. Problems arising from their inherent toxicity have, however, precluded human use. Here the authors describe findings which demonstrate that the non-toxic B-subunit of Etx (EtxB) is a highly potent mucosal adjuvant capable of potentiating protective immunity to viral infection. The mechanisms which underlie this activity arise from an ability to trigger specific signaling processes in lymphocyte populations which modulate differentially their activation, differentiation, and survival. The elucidation of these properties has led to the further use of EtxB as an agent capable of preventing the establishment of autoimmune diseases. The basis for these activities and their potential applicability to human therapies are discussed.

L35 ANSWER 12 OF 15 CAPLUS COPYRIGHT 2002 ACS
1999:36136 Document No. 130:219334 Differential activity of cholera toxin and E. coli enterotoxin: construction and purification of mutant and hybrid derivatives. Rodighiero, C.; Aman, A. T.; Lencer, W. I.; **Hirst, T. R.** (Department of Pathology and Microbiology, School of Medical Sciences, University of Bristol, Bristol, BS8 1TD, UK). Biochemical Society Transactions, 26(4), S364 (English) 1998. CODEN: BCSTB5. ISSN: 0300-5127. Publisher: Portland Press Ltd..

AB To det. whether the differential toxicity of cholera toxin (**Ctx**) and Escherichia enterotoxin (Etx) lies within the A- or B- subunits of the mols., chimeras have been engineered which comprise portions of the A-subunit of **Ctx** complexed with the B-subunit of Etx and vice versa. A mutant cholera toxin in which the C-terminal ER retention signal (KDEL) was substituted for RDEL found in Etx, was also prepd. Here the authors describe the genetic construction of mutant and hybrid toxins and a method for their purifn.

L35 ANSWER 13 OF 15 CAPLUS COPYRIGHT 2002 ACS
1999:36021 Document No. 130:164241 Receptor mediated apoptosis of CD8+T cells by the B subunits of cholera-like enterotoxins. Pitman, Richard S.; **Hirst, Timothy R.**; Nashar, Toufic O.; **Williams, Neil A.** (Department of Pathology and Microbiology, School of Medical Sciences, University of Bristol, Bristol, BS8 1TD, UK). Biochemical Society Transactions, 26(4), S338 (English) 1998. CODEN: BCSTB5. ISSN: 0300-5127. Publisher: Portland Press Ltd..

AB Heat-labile enterotoxin (Etx) B subunit (EtxB) and cholera toxin (**Ctx**) B subunit directly mediate apoptosis of CD8+T cells through an interaction with GM1, present on lymphocyte cell surfaces. Although the precise signaling pathways which mediate EtxB induced cellular activation and apoptosis remain unknown, it has been demonstrated that resp. levels of ceramide and MAPK (mitogen-activated protein kinase) activity remain unaltered in both T and B lymphocytes upon addn. of EtxB, thereby excluding a role for these signaling mechanisms.

L35 ANSWER 14 OF 15 CAPLUS COPYRIGHT 2002 ACS
1997:181160 Document No. 126:170385 Therapeutic agents and autoimmune

diseases. **Williams, Neil Andrew; Hirst, Timothy Raymond** ; Nashar, Toufic Osman (University of Bristol, UK; Williams, Neil, Andrew; Hirst, Timothy, Raymond; Nashar, Toufic, Osman). PCT Int. Appl. WO 9702045 A1 19970123, 62 pp. DESIGNATED STATES: W: AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, NL, PT, SE. (English). CODEN: PIXXD2. APPLICATION: WO 1996-GB1614 19960705. PRIORITY: GB 1995-13733 19950705.

AB There is disclosed the use, as an agent in the treatment or the prevention of an autoimmune disease, of: (i) an agent having GM-1 binding activity, other than **Ctx** or Etx, or the B subunits of **Ctx** and Etx; or (ii) an agent having an effect on GM-1 mediated intracellular signalling events, but no GM-1 binding activity. These agents may also be used in the treatment of human T cell leukemia, in the prevention of transplant rejection or GVHD or in a vaccination method for vaccinating a mammalian subject.

L35 ANSWER 15 OF 15 CAPLUS COPYRIGHT 2002 ACS

1993:424413 Document No. 119:24413 Analysis of enterotoxin synthesis in a Vibrio cholerae strain lacking DsbA, a periplasmic enzyme involved in disulfide bond formation. Findlay, Gordon; Yu, Jun; **Hirst, Timothy R.** (Biol. Lab., Univ. Kent, Canterbury/Kent, CT2 7NJ, UK). Biochem. Soc. Trans., 21(2), 212S (English) 1993. CODEN: BCSTB5. ISSN: 0300-5127.

AB To investigate the events of enterotoxin biogenesis the authors used a simplified system consisting of a vibrio strain with a chromosomal **ctx** gene deletion harboring the plasmid pMMB107, which encodes only the B-subunit of cholera-like enterotoxin (EtxB). Transposon (TnphoA) mutagenesis of this strain resulted in the identification of a mutant, UKC13::TnphoA.7A (pMMB107) with a 50-fold redn. in the level of the EtxB secretion. TnphoA insertion was found to be in a gene encoding a periplasmic protein with 40% homol. to the recently identified disulfide bond-forming protein (DsbA) of E. coli. To examine the role of DsbA in ExtB biogenesis, the dsbA::TnphoA mutant strain was cultured in minimal medium, pulse-labeled with 35S-Met and the fate of radiolabeled ExtB in periplasmic and medium fractions analyzed by SDS-PAGE and autoradiog. This demonstrated that EtxB was exported to the periplasm in both the mutant and the wild-type strain, but only secreted to the medium in the wild-type strain. The EtxB in the periplasm of the mutant strain was rapidly lost, probably as a result of proteolytic degrdn. This demonstrates that DsbA is not required for translocation of ExtB to the periplasm, but plays an important role in subsequent steps of toxin formation.

=>

---Logging off of STN---

=>
Executing the logoff script...

=> LOG Y